Practical Food Safety

Contemporary Issues and Future Directions

Edited by

Rajeev Bhat
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WILEY Blackwell

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Foreword

The food system is becoming more international at an increasingly rapid pace. Demand for fresh produce out of season and exotic ingredients combined with perceptions of lower-cost food production outside developed economies have contributed to the growth in international food trade. This ground-breaking book explores the numerous factors contributing to food safety on a global scale. While consumers may desire a wide variety of foods at low prices, there is an alternate movement to select local foods despite possible higher costs. Editors Rajeev Bhat and Vicente M. Gómez-López have recruited an international panel of experts to address the many facets of international food safety. The first six chapters provide broad perspectives on consumer beliefs, successful food safety education programs, and product traceability. Education is critical for the success of any tracing system.

Few consumers understand that eating is not a risk-free activity. Among the potential chemical risks to human health are pesticides, heavy metals, and radionuclides that can occur in foods due to poor agricultural procedures or natural environmental contamination. Genetically modified foods may contribute pesticides by their pest-control design. The melamine scandal in China is used in this text as a case study for intentional chemical contamination of foods and the steps needed to prevent such a tragedy in the future. Many naturally occurring compounds in plant foods have health benefits, but consumers may not be aware that the same chemicals may impair growth in children or have other serious health consequences. Microbial contamination of foods is likely the foremost safety concern of today's consumers. An often-overlooked food safety issue is the acquisition of foodborne infections and intoxications during international travel. Mycotoxins represent an unseen threat to foods and feed; this potential class of contaminants can fortunately be controlled. This has been addressed and provided as a case study in coffee.

Despite media stories that depict food technology as a sinister threat to food wholesomeness, emerging technologies aim to improve future food safety. The challenge for the food industry will be to educate the public about technologies such as nanotechnology and symbiotics. Explaining the scientific basis for the benefits of these new tools in a consumer-friendly manner will be essential to prevent the public backlash that genetically modified foods have endured. Other processes used to reduce microbial contamination such as cold temperature storage, electron beams, and pulsed light are the subjects of additional chapters. The important role of packaging in food safety is not overlooked; intelligent packaging may increase consumer comfort with processed food safety. The book concludes with practical approaches for reducing foodborne illness risks in animal products.

I congratulate the editors and authors for presenting a timely summary of the scope of international food safety issues. This book is a must-read for educators, processors, and regulators.

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Preface

The past decade has seen an upsurge in global interest of various aspects pertaining towards enhancement of food safety and security. With increasing knowledge of food safety, the world is witnessing tremendous efforts in improving the well-being of mankind. A rise in food safety concerns is a direct reflection of major global awareness in agro-foods sectors in world trade. Several recommendations have been made by various governing bodies and committees to solve food safety issues, which are all mainly aimed at benefiting consumers. In the present world scenario, note that that economic loss and instability due to food safety issues can have a high impact on particular nations.

Various risk factors are involved in food safety as a wide range of commodities are involved, such as: fresh fruits, vegetables, seafood, poultry and poultry products, and meat and meat products. Rapid efforts are being made globally to develop novel environmentally friendly techniques for maintaining the quality of perishable foods and agricultural commodities. Food safety issues involve a wide array of aspects involving: food processing, packaging, transportation, microbial contamination, development and application of novel technologies for post-harvest preservation, presence of food additives and banned chemicals, functional foods, and adoption of HACCP, GAP, and GMP approaches. Apart from these, rapid changes in climatic conditions can also play a pivotal role in food safety issues. To effectively manage a food safety system, proper designing, planning, and execution of representative laws

are vital and need to be supported by new research policy inputs. New safety measures with impressive research themes are regularly proposed worldwide by government and non-government organizations and policy makers. It is therefore necessary that consumers are educated about relevant measures via use of appropriate media.

The present book was planned and designed to address the vital issues of food safety including present concerns and the practical application of laboratory- (desk-) generated knowledge. Leading experts and researchers from all over the world have contributed significantly to this book, which has a wide coverage based on emerging and urgent topics pertaining to food safety issues. As well as covering the classic topics required for food safety, this book encompasses the most recent updates, addresses emerging issues, and presents novel research findings that can influence the future world.

This multi-faceted book covers many aspects such as educating consumers (consumer perceptions and practices, food safety training, product tracing systems, global food market analysis), chemical issues (chemical measurements, protection along agri-food chain, pesticide residues and toxicity, the need for visualizing pesticide toxicity during GMO assessment, melamine contamination, heavy metal residues, radionuclides, antinutrients), application of modern preservation technologies (nanotechnology, photonic methods, intelligent packaging, cold storage, use of electron beams), microbiological issues (inactivation of foodborne viruses, use

of symbiotics, predictive microbiology), and product-specific food safety issues (poultry and poultry products, meat and meat products, mycotoxins in coffee).

We the editors thank our distinguished authors and the staff of Wiley Publishing for their vital contributions. Special thanks are due to David McDade, Senior Commissioning Editor of Wiley-Blackwell, United Kingdom for his support. We are also grateful to our individual family members for their immense support and patience, and we dedicate this book to them with much love and affection.

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Food Safety: A Global Perspective

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Summary

The safety of food supply is of global concern and requires the commitment of all countries. A major reason countries import and export food is to satisfy consumer demand. Foodborne illnesses may be linked to the consumption of foods whether grown and manufactured domestically or imported. Global food safety standards are required to ensure that food will not be injurious to health regardless of its origin.

1.1 Introduction

The safety of the food supply greatly influences consumers globally. In developed countries consumers desire, even demand, products year-round regardless of the growing season of those commodities. In order to fulfill those demands, companies source products from throughout the world. The production and processing practices in developing countries may not achieve appropriate safety levels however, placing consumers within that country and throughout the world at risk of illness through export of those commodities. Many developed countries have elaborate standards and guidelines to enhance the safety of food produced domestically. Human health problems arise when best practices are not used throughout the farm to plate continuum, regardless of where the food is produced.

A plethora of factors come into play when attempting to ensure the safety of the food supply. Food safety typically relates to ensuring that food is free of pathogenic microorganisms or chemical contaminates that can negatively impact human health. The safety of the food supply is affected by food security and food fraud. Food security is a social issue in developing countries; in an effort to meet the needs of the country, food that is marginal with respect to safety may be placed into commerce and consumed. Food fraud does not always have food safety implications; however, most cases of adulteration typically involve the addition of illegal substances to food.

Government agencies strive to ensure the safety of food through national and import monitoring programs to enforce standards. Private organizations lead by the Global Food Safety Initiative, which has five benched-marked audit schemes (Safe Quality Food, British Retail Consortium, Food Safety System Certification, International Featured Standards and CanadaGAP), are accepted internationally and have emerged to bolster consumer confidence in food supply. Ensuring safety and maintaining control of a product means that audits must also be applied to members of the supply chain. Low consumer confidence in the safety of food is not confined to developed or developing countries. For example, China is becoming a major food exporter and in recent years has established three new government agencies: the State Council Food Safety Commission, the Food Safety Risk Evaluation Committee and the Food Safety Standard Examination Committee. The changes were initiated following a litany of domestic (illegally recycled cooking oil) to international (melamine in milk powder and infant formula) food safety scares. All countries continue to develop and implement new laws and regulations, striving to keep abreast of the changing face of the food industry.

1.2   National and global food safety events

In order to gain a perspective of the state of global food safety and the direction in which it is heading, past events that have shaped government and consumer response must be considered. For the most part, many of the major food safety scares are associated with intentionally adulterated or microbiologically contaminated products.

The chemical plasticizer di-(2-ethylhexyl) phthalate (DEHP) was found in an emulsifier used in powdered yogurt mix, fruit jellies and some juices and drinks produced in Taiwan. Products containing the toxic chemical were exported throughout the world. Taiwanese food regulation prohibits the use of DEHP in food.

The Chinese melamine milk scandal occurred in 2007/08, negatively impacting human and domesticated animal health globally. Melamine and other compounds including cyanuric acid were added to the milk to give the appearance of having higher protein content when tested. In China alone, at least six infants died, 800 people were hospitalized and approximately 300,000 were sickened (Gale and Buzby, 2009; Ibens, 2009). In the United States, melamine-tainted wheat gluten and rice protein imported from China and used to make pet food caused at least 17,000 pet illnesses and 4000 dog and cat deaths (FDA, 2009). Following consumption of the contaminated food, animals developed symptoms including lethargy, vomiting, loss of appetite and ultimately death. Kidney damage was apparent in affected animals, the result of the formation of insoluble crystal forming when combining melamine and cyanuric acid.

At the opposite end of the spectrum, food safety perceptions can also be shaped by the popular press and lack of consumer knowledge. In 2012 in the US, reports that 'pink slime' was being added to ground beef resulted in a public outcry followed by United States Department of Agriculture (USDA) statements assuring the public that the product was safe (Stevens, 2012). The product is actually lean finely textured beef (LFTB) that is made from beef trimmings treated with ammonium hydroxide. The LFTB is pink in colour and has a thick viscous texture. Consumers focused only on 'slime' and 'ammonium' and perceived the product to be unsafe. The USDA Food Safety Inspection Service (FSIS) and the US Food and Drug Administration (USFDA) consider ammonium hydroxide as a 'Generally Recognized As Safe' food additive.

The safety of imported products is questioned by consumers throughout the world. Products produced using acceptable production practices in their home country may be rejected by an importing country which has stricter food safety regulations. Regulatory agencies screen imported products to ensure they meet standards of that country. The US imports approximately 80% of all seafood consumed in the US. Fish farming is a growing industry, encompassing commodities from shrimp to tilapia. Integrated fish farming is practised in some countries where, for example, poultry are

raised in structures floating on or over fish pounds. The poultry faeces drop into the water and serve as feed for the fish. The faeces may contain pathogenic bacteria that present a human health risk. Depending on production practices, antibiotics may be included in the water or feed provided to the poultry, which may precipitate the selection of antibiotic-resistant bacteria. The shipments of such farm-raised fish to the US checked by the FDA are frequently contaminated (Buzby *et al.*, 2008; Gale and Buzby, 2009).

Innovative measures are often employed to ensure safety and reduce the likelihood of human illness associated with consumption of a given commodity. In 2012, the USFDA urged restaurants and food outlets to stop selling all fresh, frozen and canned oysters, clams and mussels from South Korea since such products may have been exposed to human faecal waste and contaminated with noro-virus. The shellfish are grown in natural inlets along the southern coast of South Korea. The workers on those fish farms live on boats and were releasing sewage into the production water. In response, South Korea developed floating toilets to be used by workers on the seafood farms. In this instance, the nation's food safety agencies worked with the shellfish industry to develop methods that would improve the safety of the product, preserving the industry and export potential of the product.

1.3 Foodborne illness outbreaks: imports and exports

Depending on the type of foodborne illness outbreak, the emergence of a new food safety risk may be signalled. The large 2011 *Escherichia coli* O104:H4 outbreak that was centred in Germany resulted in more than 4000 illness, over 850 cases of hemolytic uremic syndrome and 54 deaths (Frank *et al.*, 2011). The outbreak was linked to the consumption of fenugreek sprouts; the epidemiological investigation suggested the seeds were contaminated with the pathogen which grew during sprout production. The fact that sprouts were linked to the outbreak was not

remarkable. Seed sprout production practices are conducive to the growth of enteric pathogens. The pathogen *E. coli* O104:H4 had only been linked previously to one foodborne outbreak of limited magnitude. This outbreak may represent the emergence of a new foodborne pathogen.

Approximately three decades ago in the US, a large outbreak was associated with the consumption of undercooked ground beef. The causative agent was *E. coli* O157:H7, which had not been previously recognized as a foodborne pathogen. Now *E. coli* O157:H7 is a major food safety concern in the US and globally.

A devastating *Listeria monocytogenes* outbreak occurred in the US in 2011, causing 146 cases and 43 deaths (CDC, 2012). The outbreak was linked to the consumption of cantaloupe, although no previous *L. monocytogenes* outbreaks in the US had resulted from cantaloupe. A clear determination in how the cantaloupe became contaminated was not made. However, the outbreak underscores that a food may become contaminated with a pathogen even although that pathogen may not be traditionally associated with that food.

Consumer interest in the safety of imported foods increases when outbreaks occur, even when those foodborne illness outbreaks are associated with domestically produced commodities. The importation of food continues to increase in the US and other developed countries. In 2009, imports accounted for 17% of the food consumed in the US. In the US approximately 80% of the fish and shellfish consumed is imported, while nearly 34% of fruits and vegetables consumed are imported (USDA ERS, 2012). The continued increase in imports is associated with growing ethnic diversity and consumer preference for a wider selection of food products such as premium coffee, cheeses, processed meats and tropical fruit (USDA ERS, 2012). Tropical products (bananas, cocoa, spices), olive oil and cashew nuts are nearly 100% imported since domestic-produced products is close to 0%. In the US, imports of poultry meat, eggs, milk and pork is low; indeed, only 3% of head lettuce is imported. A similar import pattern has emerged in the European Union (EU) (Jaud *et al.*, 2013).

Seafood, poultry, beef and eggs were the food categories linked to most outbreaks in the US based on analysis of 4638 illness outbreaks between 1998 and 2007 (CSPI, 2009).

In some countries imports account for the majority of food consumed; South Korea imports approximately 70% of its food products. Under these circumstances, the South Korean public is extremely anxious when food safety issues develop in countries from which they import foods. Tens of thousands of concerned South Korean citizens demonstrated when the government reversed a ban on the importation of US beef in 2008. The ban was implemented in 2003 when the US announced it detected the prion responsible for bovine spongiform encephalopathy (BSE) in beef cattle.

A ten-fold increase in the importation of seafood occurred from 1988 to 2007 in South Korea. South Korea imported seafood products from about 80 countries worldwide, with much of that seafood being produced in China (AAFC, 2011; USDA FAS, 2012). The safety of food from China is scrutinized by many countries; South Korean officials found that ink and intestines from a small octopus ('nakji' in Korean) imported from China had levels of the heavy metal cadmium above acceptable standards. These events underscore the scepticism that consumers, regardless of the country, express over the safety of imported foods.

1.4 Regulations impacting food safety

Consumers are constantly seeking new and exciting foods and foods of ethnic origin. Multi-component products, even those that are apparently simple, can have an extremely complicated supply chain. A product such as a snack mix may contain less than 10 main components (almonds, sunflower seeds, coconut, dried apricots, spices, etc.), but these ingredients may be sourced from several different countries. Those components will all have different supply chains from harvest, storage, production and transport. Contamination or adulteration could occur at any step in the supply chain of a component, placing the public at risk. Should a single component, for example dried apricots, be sourced from two countries (e.g. Turkey and Uzbekistan) then the food safety risk increases as production and processing practices in both countries must now be considered. The globalization of the food system now means that a greater number of countries are sources of food products than ever before, placing an even greater burden on the government agencies that are responsible for the inspection of imported foods.

In 2012 the US FDA (FDA, 2012b) inspected 2.3% of imported food. In determining which products to inspect, the US FDA relies on risk-based criteria and data on products and manufacturers with a history of violating US import regulations. A means to highlight food safety problems associated with imported foods is to analyse import refusals. The USDA Economic Research Service analysed FDA food-related import refusals and found that fruits and fruit products, vegetables and vegetable products, and fishery and seafood products accounted for approximately 12%, 21% and 20%, respectively, of total violations (Buzby *et al.*, 2008; Gale and Buzby, 2009). Adulteration or safety violations ranged from less severe (such as an insect in cooked soup) to immediate severe risk (such as botulinum toxin in canned foods). The study included 45,941 adulteration violations, which comprised 15.3% pathogens, 25% chemical and 59.7% other sanitary violations. The vegetables and vegetable products group had the most violations for chemical contamination, while fishery and seafood products had the most violations for pathogen adulteration.

A total of 63% of the pathogen adulteration violations were associated with *Salmonella*, with *Listeria* ranked second at 24.8%. Fishery and seafood products accounted for 67.6% (3007 of 4445) *Salmonella* violations, whereas approximately 50% of violations for *Listeria* were associated with cheese and cheese products. Most of the violations for chemical residues were associated with unregistered pesticide residues than for volatile residues that exceed US tolerance levels. In the US the Environmental Protection Agency (EPA)

licences pesticide products and establishes maximum allowable limits (tolerances) for pesticide residues in food and animal feed. Products that have a poor food safety record will more likely be subject to intensified surveillance, especially if those products originate from a country with a suspect violation record.

Similarly to the US, the EU has strict import standards. Stricter regulations have been shown to hinder the trade in seafood (Anders & Caswell, 2009). Consumer demand for seafood has resulted in a doubling of global seafood trade from 1998 to 2008. Most of the seafood is produced in developing countries, in which producers find it difficult to meet the increasingly stringent regulatory barriers imposed by developed countries. Food import refusals can result in trade deflection, generally to other high-income countries. Such deflection is not necessarily associated with product refused because of potential health violations (Baylis *et al.* 2010). Stricter EU sanitary and phytosanitary (SPS) standards may reduce the number of countries that can export to the EU (Jaud *et al.*, 2013). Meeting the initial costs to comply with the standard is difficult, but more troublesome is the recurring costs associated with sustained traceability, certification or quality inspection. Countries including Iran and Vietnam experience a disproportionate number of notifications (violations associated with imported products) compared to their relatively low import shares. The US, Canada and Norway are large exporters to EU countries, but are subject to relatively few notifications (Jaud *et al.*, 2013). The study by Jaud and her colleagues (2013) suggest that a two-tier distribution is occurring where a small numbers of suppliers dominate with a fringe of marginal suppliers. Although the portfolio of suppliers is increasing, the orders are concentrated to a few suppliers of each commodity. This has the potential to be disastrous should food safety concerns for one or more of those suppliers develop.

International efforts are required to ensure safety of the food supply. Organizations including the World Health Organization (responsible for public health), Food and Agricultural Organization (responsible for food security and some aspects of food safety) and the Codex Alimentarus commission (which supports WHO and FAO by developing standards and guidelines) function at the international level to foster food safety. Countries generally have one or more agencies involved in ensuring the safety of that nation's food supply, for example: the Republic of Korea has the Korea Food and Drug Administration and the Minister for food, agriculture, forestry and fisheries of Korea; China has the General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, China Food and Drug Administration; Japan has the Ministry of Health, Labor and Welfare and the Food Safety Commission; the United Kingdom has the Food Standards Agency; Canada has the Canadian Food Inspection Agency and Health Canada; and the EU as a whole has the European Food Safety Authority. Each country may have slightly different approaches to food safety and has established different tolerances for agents that, when found in food, may present human health risks. This variability can present significant challenges in the export and import of food.

The Global Food Safety Initiative (GFSI) was developed a decade ago. The GFSI was launched to bolster consumer confidence in the safety of food supply following a number of food safety crises. The GFSI has developed definitions of food safety requirements across the industry and the entire food supply chain. Private auditors can gain GFSI recognition through meeting GFSI benchmarks and being recognized as science-based, contemporary and rigorous. GFSI recognized schemes include Global Red Meat Standard (GRMS), Canada GAP (Good Agricultural Practices) and British Retail Consortium (BRC) Global Standards. Food processors utilize these companies to conduct audits to ensure that best industry practices are being achieved and receive certification. The GFSI benchmarked food safety schemes require food production and manufacturing plants to identify their internal risks to food safety and establish a process to mitigate, reduce and ideally eliminate those risks. A major factor in the non-conformance of companies is

failure to train new employees and failure in testing and training verification. Companies seeking to conduct business in the global market must now meet food safety standards established within GFSI guidance documents. This may be burdensome, especially for companies in developing countries that have limited resources. Tying into these stricter food safety standards are revisions and the updating of national food safety laws.

The FDA Food Safety Modernization Act (FSMA) was signed into law in the US in January 2011. Similar measures aimed at tightening food safety laws have been enacted by other countries, but this discussion will focus on the US FSMA. The law will require development and use of food safety plans, based on the Hazard Analysis and Critical Point Control (HACCP) model, throughout the food industry. The law will impact foreign food suppliers, relying on foreign supplier verification program and third-party certification for imported foods. The importer must verify that a foreign supplier has all controls in place, the same as expected of a domestic supplier. The FDA now has the authority to suspend the registration of a food facility; in essence, to effectively shut down a food facility if foods produced present a reasonable probability of causing illness or death if they are consumed. Under the law, the FDA will also have the task of defining which facilities and foods fall into the high-risk category.

The framework has been established under section 201 of FSMA and the newly created section 421 of the Food Drug and Cosmetic Act. The costs associated with implementation of this type of inspection program are not trivial. A 2012 report released by FDA indicated that costs associated with inspection of domestic high-risk and non-high-risk food facilities was $21,100 and 14,200, respectively, per inspection (FDA, 2012b; http://www.fda.gov/food/guidanceregulation/fsma/ucm315486.htm). The costs increase to $24,800 per inspection of foreign high-risk food facilities. Collectively, the FSMA and other existing laws should increase the safety of food produced in the US for domestic use and export and the safety of imported foods. The FDA has established offices in countries exporting to the US to inspect facilities overseas. The FDA now maintains offices throughout the world including, but not limited to, three offices in China (Beijing, Guangzhou and Shanghai), Italy (Perma), Chile (Santiago), Costa Rica (San Jose), South Africa (Pretoria) and India (Mumbai and New Delhi) (http://www.fda.gov/International Programs/FDABeyondOurBordersForeignOffices/ default.htm).

1.5   China's food safety growing pains

China's food manufacturing sector and growth as a food exporter has increased dramatically in the past decade, presenting significant challenges for China's regulatory agencies. Indeed, Chinese food safety is a significant issue for the Chinese people and the rest of the world. The food safety issues encompass both chemical (melamine) and bacterial (*Salmonella*) hazards affecting the Chinese people and consumers throughout the world. A 2011 report estimated that in China more than 94 million cases of bacterial foodborne illness occur each year, resulting in approximately 3,400,000 hospitalizations and 8500 deaths annually (Mao *et al.*, 2011). China is now the third-largest source of imported food and aquatic products in the US, and a leading exporter of those products around the world (Acheson, 2007; Becker, 2008). This has lead to greater foreign scrutiny of China's food safety and pressure to conform to international standards. The spotlight on China's food safety problem lead to the enactment of the Food Safety Law (FSL) in 2009 by the Chinese government. The Food Safety Law replaced the outdated Food Hygiene Law, but the law is only as good as the measures taken to ensure that it is implemented. Notwithstanding, the FSL contains measures designed to prevent and eliminate future food safety problems. More specifically, the law provides a starting point for a new regulatory scheme governing food safety: increased inspections, mandatory recalls and a risk-based approach to determining foodborne illness threats. The Chinese administrative authorities that have

the responsibility of implementing the FSL now need to be held accountable for enforcement or lack thereof to the public and through the Chinese legal system.

The number of incidents of food contamination and adulteration in Chinese food imports are the main reason why such a focus is placed on the safety of food from China. As indicated previously, China is one of the fastest-growing sources of US food imports; however, less than 1% of the US food supply comes from China. China is a major supplier of fish and seafood (predominantly farm raised), juices and canned fruits and vegetables (Gale and Buzby, 2009). Approximately 60% of US apple juice supply and more than 50% of the garlic supply in 2007 were imported from China. Safety concerns arise as more knowledge of China's production, manufacturing and handling of food becomes available. Crops have been found to contain unacceptable (based on US tolerances) levels of pesticides and heavy metals, and animal products found to contain veterinary drugs. Fresh produce, which is typically maintained in a cold chain from harvest to retailer in the US, may be transported in open trucks, increasing the risk of contamination. Understanding food microbiology will assist in reducing the cross-contamination of commodities during handling. Manufacturing ice from non-potable water and then using that ice to chill fresh fruits and vegetables or seafood can result in cross-contamination of those commodities. Although China has many large modern farms and manufacturing facilities, there also exist millions of small establishments that lack the technical expertise and resources to develop and implement modern practices designed to ensure the safety of food destined for domestic consumption and potentially export.

China has dealt with many scandals concerning domestic and exported food products, but is also increasing measures to ensure the safety and quality of food being imported by China. Notably, in 2012 products produced by Kraft (cream cheese), Nestle (chocolate bars) and Ikea (chocolate almond cake) were destroyed by the Shanghai Quarantine Bureau. In the case of the chocolate almond cake, excessive levels of coliform bacteria were found associated with the product. An increase in these types of actions is likely as the Chinese regulatory authorities seek to improve the safety of domestic and imported foods.

1.6   Food safety and product testing

The safety of imported and exported food is based on food standards and regulatory limits. Global sourcing means that exported products are tested to show compliance and imported products are tested to check compliance. Testing is generally performed to determine biological hazards (parasites, bacteria, viruses or bovine spongiform encephalopathy) or chemical hazards (veterinary drugs, pesticides, natural toxicants or adulterants). Sensitive and rapid test methods are required so that products, particularly those that are highly perishable, can be evaluated and moved into the food supply if deemed safe. Even with a robust toolbox of testing methods, intentional adulterants and new emerging pathogens may fail to be detected. For example, testing designed to determine the presence or absence of a given microbe such as *Escherichia coli* O157: H7 would fail to detect *E. coli* O104:H4. Products contaminated with either pathogen present a human health risk. New laboratory technologies must therefore be developed that are rapid and detect a range of microbial agents (Cowan-Lincoln, 2013).

1.7   Fresh fruits and vegetables safety

The safety of fresh and fresh-cut fruits and vegetables has received considerable attention globally. The FSMA in the US now requires the US FDA to establish science-based minimal standards for the safe production and harvesting of those types of raw fruits and vegetables (e.g. lettuce, tomatoes and cantaloupes) for which standards are necessary to minimize the risk to human health including death. The 'Standards

for growing, harvesting, packing, and holding of produce for human consumption' will focus on microbiological hazards (FDA, 2013b). The reason for this is that illnesses attributed to chemical hazards associated with the consumption of fresh and fresh-cut produce are rare (FDA, Food and Drug Administration, 2013a). Similarly, data show that between 1997 and 2011 there were no Class I recalls of produce associated with physical hazards (FDA, Food and Drug Administration, 2013a) (Class I recalls are initiated when there is a reasonable probability of the product causing serious health problems or death).

The microbial safety of produce is a concern; between 1996 and 2010 approximately 23% of total outbreaks of foodborne illness were produce related. Imported produce and domestic produce were identified as vehicles in these outbreaks. A wide range of products was associated with those outbreaks including green onions, cantaloupe, spinach, blueberries and lettuce. The majority of the outbreaks were however associated with sprouts and leafy greens. Bacterial agents (e.g. *Salmonella, L. monocytogenes* and *E. coli* O157:H7) were associated with 86.5% of outbreaks followed by parasites (11.6%) and viruses (1.9%). Under the FSMA, foreign suppliers are required to meet the same standards as domestic producers. This also includes foreign farms that meet the criteria of 'covered farms' that grow, harvest, pack or hold covered produce for import into the US. Meeting the standards outlined will likely be costly and may limit the ability of some developing countries to comply, impeding exports of fresh fruits and vegetables to the US.

1.8   Conclusions and future outlook

The increase in the breadth and stringency of food safety regulations will only enhance the safety of the food supply if those regulations are enforced. The lack of appropriate infrastructure and well-trained inspectors, particularly in developing countries, will hinder improvements in food safety. Industry and government cooperation, both domestic and international, is key to facing the challenge of food system protection; failure will have a negative effect on human health. As the global integration of the food supply continues to increase, the focus must remain on providing consumers with safe food regardless of its source.

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2
Food Safety: Consumer Perceptions and Practices

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Summary

We describe the knowledge, attitudes and behaviors of consumers regarding food safety, as well as social, cultural, economic and demographic factors that influence consumer attitudes and behavior. Consumer perspectives associated with the benefits and risks of technologies such as irradiation, genetic modification, nanotechnology, use of hormones in food animals and organic foods are discussed. In recent years, substantial resources have been devoted to increasing the level of food safety knowledge among consumers. The emphasis of such consumer education should be placed on behaviors that have the greatest impact on reducing risk of foodborne illness.

2.1   Introduction

Consumers have a wide range of choices among foods that are competitively priced based on their quality attributes. Consumers look for qualities that meet their needs. Aesthetic, organoleptic and healthful qualities, as well as the product and brand image, influence their choices. In addition to the food meeting their quality preferences, consumers expect it to be intrinsically safe (Grunert, 2005; Lobb, 2005; Verbeke *et al.*, 2007; van Rijswijk and Frewer, 2008).

Foodborne diseases affect millions of people each year. The World Health Organization identified that the burden of foodborne disease is largely unknown, so initiated a collaborative effort to assess the worldwide foodborne disease problem. This includes diseases associated with biological and chemical contaminants (WHO, 2012).

Some countries have produced their own estimates. In England and Wales, 1.7 million cases of domestically acquired foodborne diseases are estimated to occur annually (Adak *et al.*, 2005). In the United States, an estimated 9.4 million domestically acquired cases of foodborne diseases are caused by 31 major pathogens (Scallan *et al.*, 2011a) and 38.4 million are attributed to unspecified agents (Scallan *et al.*, 2011b). The Public Health Agency of Canada estimated that 4 million cases of domestically acquired foodborne disease occur each year in Canada; 1.6 million of these are caused by 30 pathogens and 2.4 million incidents of acute gastrointestinal illness are attributed to unspecified agents (Thomas *et al.*, 2013). These statistics more accurately represent the estimates of acute gastrointestinal illnesses per year in the United States (Mead *et al.*, 1999) and in Canada (Thomas *et al.*, 2008).

Difficulties in making accurate estimates include the under-reporting of known food-related diseases, the transmission of food-related pathogens through non-food sources and unrecognized agents in food (Wilcock *et al.*, 2004). Furthermore, long-term health effects are not captured in these estimates. In addition, illness or disease may stem from chemical contamination such as allergens, naturally occurring toxins (e.g. mycotoxins), industrial pollutants (e.g. dioxin), agricultural chemicals (e.g. pesticides and antimicrobials) or other compounds.

The consequences of a contaminated food supply, as well as inadequate hygiene and food handling practices that increase food risks, are substantial. The economic burden of foodborne disease can be associated with medical costs related to consumer illness and death, productivity loss, loss of customers and sales and associated lawsuits (Buzby *et al.*, 1996). Economic costs are also associated with loss of consumer confidence in the food industry, lost production (Wilcock *et al.*, 2004; Lewis *et al.*, 2013) and, potentially, lost jobs. 'Hidden' costs include pain and suffering, time spent by caregivers and travel-associated costs for those affected and their caregivers (Nyachuba, 2010). The 2008–2009 *Salmonella* Typhimurium outbreak associated with peanut

products produced by the Peanut Corporation of America, for example, led to 716 identified illnesses and 9 deaths in 46 US states and Canada, and an international recall of 3918 peanut butters and peanut-butter-containing foods manufactured by various companies (CBC, 2009; Cavallaro *et al.*, 2011). Production facilities were shut down. Subsequently, four former officials of the bankrupt company were criminally charged, and $12 million was awarded to the victims (Goetz, 2013).

Governments at various levels have recognized the risk and potential impact of food contamination. In Britain, the 1990 Food Safety Act and the 1995 General Hygiene Act significantly affected the food safety risk management practices in the food sector, shifting the focus from fraud prevention to a proactive scientific-based food safety approach (Sockett, 1991). The Food Standards Agency, created in the UK in 2000, included a mandate to conduct retail food surveillance and coordinate food safety research (Tent, 1999). Soon after, the European Parliament enacted the General Food Law Regulation which established the European Food Safety Authority and required member countries to adapt their laws by 2007 to comply with Regulation EC/178/2002 (European Commission, 2002). In the United States in 1997, then President Clinton launched a National Food Safety initiative to enhance surveillance, improve risk assessment, inspection and compliance, educate consumers and conduct important new research (Tent, 1999). Food safety standards have continued to improve with the introduction of various rules and guidance documents, as well as the Food Safety Modernization Act brought into force by President Obama in 2011 (USFDA, 2013).

Reforms introduced with the latter legislation focus on preventing contamination of the food supply, rather than reacting to contamination issues (USFDA, 2013). Similarly, the Government of Canada, responding to recommendations put forth in the Weatherill Report following a nationwide listeriosis outbreak in 2008, passed the Safe Food for Canadians Act in 2012. This statute brings together several pieces of legislation to strengthen the oversight of food that is subject to federal laws, thereby enhancing the safety of

the food supply (CFIA, 2012). Many other countries have adopted legislation to protect consumers and the food supply. Recently, China consolidated food safety standards and introduced more than 300 new standards through the China National Center for Food Safety Risk Assessment. Plans are to introduce a single set of food safety standards by 2015 (Xiaodong, 2013).

The implementation of regulations, good manufacturing practices (GMP) and hazard analysis critical control point (HACCP) systems in processing facilities are essential to reduce the risk of biological, chemical and physical contamination. As consumers are unable to make a clear distinction between food quality and safety, when they perceive product quality they expect the food to be safe (van Rijswijk and Frewer, 2008). Despite consumers' expectations, zero food risk is not however possible (Lobb, 2005).

Consumers' attitudes and practices related to food safety are themes of interest to food producers and retailers, public authorities and health educators. This interest has been reflected in discussions about how food safety should be defined and how consumers perceive food safety and choose food. This chapter provides an overview of consumer perceptions about what constitutes safe food and safe food handling practices. It is an update of an article by Wilcock *et al.* (2004), expanding the section on novel technologies

and hormone use and adding information about deliberate and accidental food contamination. Moreover, we discuss the relationship between consumer perception, food risk and the impact on attitude and practices.

2.2 Novel technologies and issues

A safe food is food that is free of all hazards. Henson and Traill (1993) suggest food safety can be viewed as the inverse of food risk, that is, the probability of not experiencing a negative health outcome from consuming a food. Consumers may see it simply as food that is harmless to health (Jevšnik *et al.*, 2008). Novel technologies used in food production and processing can be introduced to the market after government approvals that consider food safety and other factors. Taking different approaches to risk analysis, some governments may not approve controversial technologies that other governments accept. Moreover, some consumers may mistrust a technology or may hold attitudes or philosophical perspectives that lead them to reject some foods that other consumers will choose. An overview of consumer concerns about controversial technologies is provided in Table 2.1. When examining consumers' attitudes towards food safety, it is important to consider

Table 2.1 Highlights of consumer concerns about novel food technologies.

Technology	Consumer concerns	Food safety
Irradiation (to reduce pathogens and pest contamination, increase shelf-life)	Safety, environmental safety	Low risk, permitted use varies among countries
Hormone supplementation for food animals (to aid livestock production)	Safety, lack of consumer benefit	Assessment of risk mixed, permitted use varies among countries
Genetic modification (to create desired traits in plants and animals through genetic manipulation such as cross-breeding, mutagenesis or biotechnology)	Safety, potential negative social and economic outcomes, potential negative environmental outcomes, consumer choice/consent	Assessment of risk mixed, permitted use varies among countries, food labeling varies among countries

(Continued)

Table 2.1 (Continued)

Technology	Consumer concerns	Food safety
Genetic engineering, an aspect of genetic modification (to create desired traits in plants and animals through genetic manipulation involving recombinant DNA or genetic engineering techniques)	Safety, potential negative social and economic outcomes, potential negative environmental outcomes, consumer choice/consent	Some transgenic plants approved for food and/or feed in some countries, transgenic animals not approved for use
Nanotechnology (to enhance food characteristics such as safety, quality, structure, taste, texture, color and nutrition)	Consumer benefits versus risks, potential negative health and environmental outcomes, unknown outcomes, consumer choice/consent	Risks largely unknown, gaps in knowledge and regulatory frameworks for oversight

attitudes towards controversial technologies and issues that may affect foods.

2.2.1 Irradiation

Irradiation is a technology discovered a century ago when an ionizing radiation process to improve food quality was patented in the United Kingdom (O'Bryan *et al.*, 2008). In 1983, the Codex Alimentarius Commission recognized the safety and viability of irradiation for foods; the World Health Organization (1994, 1999) confirmed their safety and wholesomeness. Report after report emphasizes the effectiveness of irradiation: the process of exposing food to a carefully controlled amount of ionizing energy to reduce the microbial risk associated with raw and minimally processed foods including meat, seafood, fruit, vegetables, grains and spices. It is also used to reduce spoilage or sprouting.

Molins *et al.* (2001) equate the public health value of irradiating foods to that of thermal pasteurization of milk, which is mandatory in many countries. Irradiation, sometimes referred to as cold pasteurization, can effectively destroy bacteria and parasites, but not viruses (O'Bryan *et al.*, 2008). With *Campylobacter* and *Salmonella* as two of the top agents causing foodborne disease in the US, Canada and parts of the United Kingdom (Adak *et al.*, 2005; Scallan *et al.*, 2011a; Thomas *et al.*, 2013), irradiation

of poultry products alone has the potential to considerably reduce the associated public health and economic burdens.

Quality concerns formerly associated with irradiated meat, resulting from oxidation and the production of toxic compounds, have been reduced by adjustments in temperature, additives (such as vitamin E) and atmosphere during irradiation. Sensory panels have been unable to distinguish between irradiated and non-irradiated meats, or the irradiated meat was identified but maintained an acceptable quality (O'Bryan *et al.*, 2008). Additionally, with food safety management systems commonly required in processing establishments, consumers ought to be less concerned now than in the past about the potential use of irradiation technology to compensate for poor product quality and/or poor manufacturing and unhygienic practices. According to Brewer and Rojas (2008), more than one-third of US consumers had no to low concern about irradiated foods (17.7% and 21.1%, respectively), while nearly as many were strongly or very strongly concerned (19.8% and 17.5%, respectively). Interestingly, this represents a shift of consumer perception in an earlier study (Brewer and Prestat, 2002) from having no/low concern to being moderately concerned. The reason for this shift is not clear, although overall consumer confidence in the food they eat decreased by 10%.

Consumers who were given information on the irradiation process and participated in market trials of irradiated products were much more likely to accept this technology. In 1991, a small food store in Chicago held one of the most successful trials in the US. Irradiated strawberries, oranges and grapefruits outsold the non-irradiated fruits by a ratio of 9:1. The following season, irradiated strawberries outsold non-irradiated strawberries by 20:1. This phenomenon encouraged approximately 60 stores in Indiana, Illinois and Ohio to sell a variety of irradiated foods (Bruhn, 1995).

Studies by Bruhn (1995, 1998) consistently show that a high percentage of consumers who are informed about the science will prefer irradiated foods. Consumers in focus group discussions identified three important messages: the safety and wholesomeness of irradiated food, the effectiveness of the process to destroy bacteria and protect against foodborne illness and the safety endorsement by health authorities (Bruhn, 1998). Statements from the American Dietetic Association and the American Medical Association demonstrate professional support from credible sources that have an interest in public health. These organizations see their role as assisting in the education of consumers about the technology (IFST, 1999). As such, they can help to educate consumers on the advantages and limitations of the technology to enable consumers to make informed and rational decisions about buying and eating irradiated food (Bruhn, 1998).

Despite consumers showing positive response about irradiated foods when given information from scientific sources, Fox et al. (2002) identified that negative information reduced the effect. When consumers were given positive expert information balanced with negative information from consumer advocacy groups, the positive effect of the expert messages was lost. The reason for the reduced effect is not clear, although it is plausible that consumers give an element of 'expert' status to consumer advocacy groups when faced with conflicting information. Fox et al. (2002) suggest their findings may be applicable to novel technologies such as genetic modification.

2.2.2 Genetic modification

Genetic modification of foods remains a controversial issue among consumers. Much of the concern appears to relate to genetic manipulation that involves recombinant DNA or biotechnology techniques associated with genetic engineering. Genetic engineering of plants and animals is made possible by molecular biology techniques that enable the transfer of genes from one organism to another. In 2010, 22% of the seeds marketed and planted around the world were genetically engineered. The crops included those that resisted insects, disease, pesticides and extreme weather conditions, and/or had enhanced nutritional value compared to conventional breeds (Benessia and Barbiero, 2012). Neither the genetically engineered salmon nor the Enviropig, the first transgenic animals nearing commercialization, have yet made it to market (Leeder and Leung, 2013). While numerous studies have shown genetically engineered plants to be safe, others have identified potential health risks and raised questions about experimental design and long-term effects (de Vendômois, 2010; Domingo and Giné Bordonaba, 2011).

Using a relatively simplistic approach, Brewer and Rojas (2008) found that 31.7% of consumers would not purchase genetically modified foods. In fact, 22.6% of consumers believed that genetically modified foods were not safe to eat under any circumstances. Furthermore, 39.3% would pay more for non-genetically modified foods. These findings do not provide much insight into the rationale for the responses. While there may be some concerns about the safety of genetically engineered foods and ingredients, consumers seem to consider other factors when considering the acceptance of food biotechnology (Kuzma and Besley, 2008).

Several studies have considered consumer perceptions of genetically modified foods in different countries (Mucci and Hough, 2004; Christoph et al., 2008; Costa-Font and Gil, 2009; to name a few). Research on this topic requires looking beyond food safety and asking questions that will enable the assessment of complex interactions among socio-psychological factors affecting the acceptance of genetically modified foods.

Variables may be related to benefits, risks/costs, uncertainty, trust and/or acceptability. According to Siegrist (2008), benefits and/or demonstrated experience (e.g. tasting) tended to increase the likelihood that consumers would accept genetically modified food. This is supported by de Liver *et al.* (2005) who determined the relative importance and independence of positive and negative thoughts (e.g. (un)useful), positive and negative feeling (e.g. pleasure/pain) and risk perception (e.g. risk/worry) and their influence on overall attitude towards the technology.

A 'natural' or 'traditional' product may taste better than the same product labeled as made with genetically modified ingredients. In fact, valuing 'naturalness' and 'organic' foods were important indicators of a negative perspective about new technologies (Siegrist, 2008). As correlations, however, one might wonder whether the natural or organic preference was established before a negative attitude to the technology.

Labeling of genetically modified foods is voluntary in the US and Canada and mandatory in Europe. According to Brewer and Rojas (2008), 78.4% of US consumers agreed that foods containing genetically modified components should state so on the labels. In contrast, Hoban (1998) indicated that three-quarters of American consumers supported existing labeling legislation that biotechnology products only need labeling if they have changed in a substantive way. Labeling provides the opportunity for choice and informed consent. Consumers may prefer to avoid genetically modified components for philosophical or any other reasons. Without a label statement, they would have to seek out and purchase products with a certification standard that indicates absence of genetically modified components. Reiterating a science-based risk assessment that claims a genetically modified food is safe, or dismissing consumer concerns, seems patronizing. Furthermore, it ignores the ethical principles relating to the individual, such as autonomy (Kuzma and Besley, 2008).

Siegrist (2008) identifies that trust is important: trust in the source of information about the technology, in the people delivering the message and in the industry. The environmental, economic and other risks of introducing a transgenic species into production may have been downplayed. Benessia and Barbiero (2012) refer to the myths of containment and enhanced yield. Furthermore, recent experiences with cross-pollinated plants have shown that genetically modified crops are not always contained, giving credence to non-food safety concerns (Biello, 2010). Consumers may have legitimate concerns about some risks about genetically modified foods.

2.2.3   Nanotechnology

As with any new technology, nanotechnology brings with it both benefits and risks. The technology is so new that there are very few studies on consumer knowledge about it or attitudes towards it. Although nanotechnologies can vary widely, they have certain characteristics in common. It is these characteristics upon which the US Environmental Protection Agency based its definition: 'Nanotechnology is research and technology at the atomic, molecular or macromolecular levels using a length of scale approximately 1 to 100 nanometers, in any direction; the creation and use of structures, devices and systems that have novel properties and functions because of their small size; and the ability to control and manipulate matter on an atomic scale.' A study by Gaskell *et al.* (2005) concluded that 'the US has a more supportive culture for the adoption and development of nanotechnology than Europe'.

In recent years, nanotechnology has been used in food and food packaging and it is claimed that the safety and quality of food will improve as a result. In a recent exploratory study that investigated the acceptance of nanotechnology in food, a 'one-to-one deliberative discourse' was conducted between consumers who were unfamiliar with the use of nanotechnology in food and a food scientist with expertise in nanotechnology. The food scientist presented each consumer with a series of hypothetical scenarios about the benefits and risks of applications of nanotechnology in food. In-depth interviews with the consumers were conducted before and after the scenarios in order to

determine their perceived influence on attitudinal change. Information presented in the scenarios seemed to have a positive impact on consumers' attitudes toward the use of nanotechnology in food and the likelihood of consumers purchasing foods that used nanotechnology either during processing or packaging. There was greater acceptance of the technology when the consumers perceived that the benefits (to themselves or society) outweighed the risks (Greehy, 2011).

A review article on new food technologies presented a case study which included consumer attitudes towards nanotechnology (Rollin *et al.*, 2011). The highlights of this report include the following: European and North American consumers were equally optimistic about the future of nanotechnology; Europeans were more concerned about its impact on the environment and less confident in regulation; benefits of the technology, fear of the unknown and the ability of regulators to ensure nanotechnology safety were the main risks perceived by consumers; and, demographically, women were substantially less optimistic and slightly less supportive of the technology than men. Interestingly, it was suggested that religion may influence consumer perceptions of the relative benefits and risks of nanotechnology.

2.2.4   Hormone use in food animals

The European Union has prohibited hormones as production aids since the early 1980s (European Commission, 2005). In contrast, Canada allows hormone use only in beef cattle, while the US permits hormone use for beef cattle as well as for milk production in dairy cattle. For context, hormones are naturally present in all mammals with varying levels depending on age, physiological status and pregnancy status (Waltner-Toews and McEwen, 1994; Raun and Preston, 2002). According to Brewer and Rojas (2008), nearly half the consumers in a US study were concerned about 'hormone residues in poultry, meat or milk', which measured as strong and very strong concerns (24.5% and 20.9%, respectively) on a 5-point

Likert scale. This was an increase in concern from the previous study in which Brewer and Prestat (2002) found consumers strongly or very strongly concerned about hormone residues in meat (17.1% and 21.9%, respectively) and in milk (17.6% and 18.2%, respectively).

2.2.4.1   Beef production

The natural hormones used as production aids for beef animals include testosterone, estradiol-17β (estrogen) and progesterone; these are identical to those produced by humans. Synthetic hormones mimic the naturally occurring chemicals (Doyle, 2002). These hormones increase the weight gain and feed efficiency of steers and heifers raised for meat (Waltner-Toews and McEwen, 1994; Doyle, 2002). When natural hormones are used to treat animals, the levels in the animals' systems remain within the normal range of untreated animals so no maximum residue limit is established (Doyle, 2000). Synthetic hormones are treated differently from natural hormones. They are considered contaminants at any level (Waltner-Toews and McEwen, 1994), and require safety evaluations to examine their toxicological effects on animals (Doyle, 2002).

Most exogenous hormones are in slow release form, implanted in the ears of beef animals (Waltner-Toews and McEwen, 1994; Doyle, 2002). The implants increase hormone levels in the animal tissues; however, the ears would have the highest amount of residue and are discarded at slaughter. According to Waltner-Toews and McEwen (1994), residues of the synthetic zeranol, which mimics estradiol, have been detected in beef liver up to 120 days after having been implanted; the withdrawal period is 65 days. Improper use of the hormones such as excessive (UKVPC, 2006) or incorrect implantation may increase exposure of humans to these chemicals, as would illegal use in non-beef animals such as veal (Waltner-Toews and McEwen, 1994). The European Union cited a number of veterinary drug studies that either support the claim that sex hormones for beef production are unsafe or demonstrate that the science is uncertain. In addition, illegal use of hormones can be a concern:

violative residues are occasionally found in animal products (Smith *et al.*, 1997).

Despite the risk assessments that showed low risk and re-evaluation of data, the European Union's Scientific Committee on Veterinary Measures Relating to Public Health (SCVPH, 2002) expressed concern in 1999 about what it considered a substantial body of research suggesting that estradiol, a natural hormone, is a carcinogen. In 2003 the European Parliament amended its directive that prohibited using hormones for growth promotion, and significantly reduced the circumstances under which estradiol is permitted for therapeutic use in food animals (European Commission, 2005). In contrast, the United Kingdom's Veterinary Products Committee (UKVPC, 2006) indicated that there was more than enough evidence to show that estradiol poses no risk to humans unless the area at the implant is consumed. UKVPC confirmed that, despite some unknowns, the health risk from meat treated with growth-promoting hormones is low.

2.2.4.2   Milk production

Bovine somatotropin (BST) is a growth hormone in cattle that can be used to increase milk production in lactating dairy cows by 10–15% during treatment (Crooker *et al.*, 1994). Recombinant DNA technology has made it practical to produce synthetic BST (rBST) commercially. The rBST molecule has essentially the same chemical structure and biological activity as BST (Crooker *et al.*, 1994; IFST, 2004). Cows treated with rBST produce milk that is considered to have the same composition of nutrients and hormones as milk produced by untreated cows (FAO, 1993; Crooker *et al.*, 1994). rBST is a large protein so is digested before its molecular components are absorbed in the gut (Crooker *et al.*, 1994; Waltner-Toews and McEwen, 1994). The molecular structure of BST is different from human somatotropin, making it unable to bind on human receptor sites and rendering it inactive as a growth hormone (Crooker *et al.*, 1994; IFST, 2004). Even if rBST were to be consumed by humans in milk or meat, it could not be absorbed or bound to receptor sites.

While BST and rBST levels do not increase in milk of treated cows, researchers have found a significant increase in the amount of insulin-like growth factor I (IGF-I). IGF-I is normal and highly variable in milk (FAO, 1998). Bovine IGF-I is identical to human IGF-I and, unlike rBST, is not readily denatured at pasteurization temperatures (FAO, 1993). In its risk assessment, the Joint FAO/WHO Expert Committee on Food Additives (FAO, 1998) noted that human babies are exposed to equal or higher levels of IGF-I in breast milk than is found in bovine milk from rBST-treated cows. The Expert Committee determined that the impact, probability and uncertainty related to a negative human health outcome from rBST and bovine-source IGF-I are low. As a result, there can be no specified acceptable daily intake (ADI) and maximum residue level (MRL) for these compounds.

Some consumers expressed concern about the food risk associated with an expected increase in antimicrobial use due to increased mastitis in heavy producing cows (Kaiser *et al.*, 1992). This has not been substantiated (FAO, 1998).

2.2.4.3   Benefit versus risks

The main benefit of the use of hormones is the increase in production efficiency. This is a benefit to producers, although consumers may benefit through product pricing (Kaiser *et al.*, 1992). Is there a food risk worth taking for this potential benefit? Some countries identified non-food outcomes to consider (e.g. negative animal welfare risks) in the balancing of issue risk management. According to Brinckman (2000), the EU justification for banning rBST was finally associated with animal health and welfare concerns rather than a precaution related to food risk. The Government of Canada also cited animal health and welfare concerns in its decision not to permit the use of rBST.

Risk communication may help mitigate some of the concern. In a premarket survey, Kaiser *et al.* (1992) identified that consumers who had negative attitudes towards the use of rBST were more likely to want milk produced with it to be labeled. The researchers suggested that the

dairy industry should implement an educational strategy to counteract negative consumer attitudes toward rBST use in milk production. Kolodinsky (2008) identified that a negative attitude to genetic modification influenced consumer evaluation of milk labeled as rBST-free and organic.

2.2.5 Organic foods

Organic food is produced using environmentally sustainable methods that do not involve inputs such as chemical fertilizers, most pesticides, genetically modified seeds/feeds, hormones or antibiotics. Foods must not be processed using irradiation, not contain genetically modified organisms and not contain industrial solvents or chemical food additives (Paul and Rana, 2012). Individual philosophical perspectives inspire people to produce, purchase and/or consumer organic products.

Who purchases organic food? An examination of demographic variables suggests that younger consumers generally have more positive attitudes towards organic food (Magnusson et al., 2001) but it is older and more highly educated consumers with higher household incomes who tend to purchase it more frequently (Paul and Rana, 2012; Roitner-Schobesberger et al., 2008; Sangkumchaliang and Huang, 2012). A factor which restricted the market share for organic vegetables in Thailand was the difficulty that consumers experienced in differentiating between 'pesticide safe' labels and organic produce labels (Roitner-Schobesberger et al., 2008).

The most commonly reported reason for the purchase of organic food is that it is healthier; consumers want to avoid the pesticides that are used in conventional food production because they perceive them to cause health problems in the short or long term. While organic fruit and vegetables should contain fewer pesticide residues than their conventionally grown counterparts, the difference has been reported to be insignificant (Magkow et al., 2006). Some leafy, root and tuber organic vegetables seem to contain lower levels of nitrate than conventional vegetables, but it is unclear whether nitrate in the diet is a threat to human health (Magkow et al., 2006). Consumers should therefore not equate the term organic with food safety.

A second motive that has been reported for purchasing organic food is that the taste is better. This motive has however been shown not to hold true for different categories of food. Concern for the environment has also been shown to influence consumers' attitudes towards organic foods, but it is of less importance than perceptions of superior healthiness and taste. Other motives underlying purchase behavior include concern for food safety, concern for animal welfare, support for the local economy, appreciation of the 'wholesomeness' of organic food, reminiscence of the past and its current popularity (Hughner et al., 2007).

Consumer attitudes towards organic foods do not translate directly into purchase behavior. According to Andersen (2007), households that perceive organic foods as healthier than conventional foods are more likely to purchase organic food and are more willing to pay a premium price for it than other households. Factors that have been reported to interfere with the intention to purchase are the lack of availability of organic food, cosmetic defects, consumer skepticism about organic food labels, lack of knowledge due to insufficient marketing/promotion and, finally, general satisfaction with conventionally grown food (Hughner et al., 2007). While there are numerous references that identify reasons for the purchase or non-purchase of organic foods, very few address which members of a household actually consume organic food.

2.2.6 Deliberate and accidental contamination

With the food supply becoming increasingly global, it is essential that it be protected against contamination that is either deliberate or accidental. Deliberate contamination is usually for the purpose of economic gain, although there has been a US report of contamination for political gain (Homeland Security Newswire, 2009 in Zach et al., 2012). There has also been documentation

of interest in contamination of the food chain by terrorist groups abroad (Kennedy, 2009 in Zach *et al.*, 2012) although, to date, such a terrorist attack has not occurred. Regardless of the underlying reasons for the contamination, the outcome can be serious or even fatal to consumers.

The range of practices designed to deliberately contaminate food includes adulteration, counterfeiting, comingling and substitution. Examples of deliberate contamination include 'watering down or adding inert ingredients to products such as infant formula and drugs, relabeling products that have passed their expiration dates, relabeling to change country of origin (e.g. honey laundering; Berfield, 2013), and substituting cheaper species of fish for more expensive ones' (US Government Accountability Office, 2009 in Zach *et al.*, 2012, p. 154). Most recently, European consumers were shocked to learn that horse meat had been substituted for beef and other meats (Premanandh, 2013). Even organic products are not exempt from fraudulent claims. The USDA maintains a list of companies that have falsely claimed to be suppliers of certified organic products.

Import of foods and of raw materials used to produce processed foods presents a particular challenge. In the latter case, the opportunity for deliberate contamination is increased because of the potential for a single ingredient to contaminate multiple products and also because of the greater length of the food chain. The more complex the food chain, the greater the opportunity for contamination. Food safety concerns became a major issue in domestic food markets in China between the years 2003 and 2006 because of incidents involving food poisoning, discovery of dangerous dyes and additives to food products, fraudulent products and the sale of food that had passed its expiration date (Wang *et al.*, 2008). In 2008, contamination of infant formula with melamine involved deliberate contamination that killed young children. While imports from China tend to be highlighted, those from more developed countries also occur. According to a recent ABC News report on counterfeit foods (ABC, 2013), 7% of the US food supply is estimated to be counterfeit. Products such as olive oil, spices,

tea, pomegranate juice and lemon juice were highlighted, but in fact counterfeiters are interested only in financial gain, so all food products are potential targets. Governance in the form of anti-counterfeit measures by food manufacturers, ongoing diligence, training by trade associations and government initiatives and regulations all support the fight against counterfeit goods.

Accidental contamination of food does occur. This may occur in imported foods because members of the food chain (farmers through food processors) are unfamiliar with specific safe practices or with additives that are approved for use. For example, non-hygienic growing, packing and shipping of cantaloupes from Mexico led to the growth of *Salmonella* and ultimately to the ban on importation of cantaloupes by the US (Zach *et al.*, 2012).

Protecting consumers from both deliberate and accidental contamination has been, and will continue to be, an ongoing challenge. Traditional food safety initiatives are no longer considered adequate to protect the food supply from deliberate attack. A variety of measures, spanning the entire food production cycle, are used. At the farm level, security cameras may be used to enhance the surveillance of entire facilities, including fields and parking facilities. At packing plants and processing facilities, HACCP programs can and should be carefully examined in order to identify and strengthen the most vulnerable points. During shipping, the opportunity for tampering and terrorism can be reduced by use of secure locks, security systems and seals. At the retail level, labels on food should provide accurate and adequate information to allow consumers to make informed choices about the food they purchase.

Hence, testing to ensure that the content of a package matches the information on the label has become a routine practice. It has been suggested that a description of issues of authenticity on products would help to increase consumer confidence in those products (Premanandh, 2013).

While consumers can be assured that food is well protected against both accidental and deliberate contamination, it must be understood that doing so

comes with a cost. All security enhancements must be balanced against the tight profit margins on food. Even with these safeguards, most consumers probably do not understand the crucial role of food safety regulations. In order to protect consumers, it is important to understand their attitudes towards food safety.

2.3 Consumer attitudes, knowledge and behavior

An attitude towards an issue is a relatively permanent and stable evaluative summary about it. Attitudes are important psychological constructs because they have been found to influence and predict many behaviors (Kraus, 1995).

2.3.1 Types of food safety issues

Consumer attitudes towards food safety can be differentiated based on the type of food safety issues of concern. Brewer *et al.* (1994) proposed that six factors dominated respondents' attitudes towards the safety of their food: (1) chemical issues such as food additives and hormones in milk; (2) health issues such as cholesterol content; (3) spoilage issues; (4) regulatory issues, e.g. food inspection and labeling; (5) deceptive practices; and (6) ideal situations, such as time required for pesticide safety assessment.

Consumer attitudes and behavior towards food have been studied using approaches such as the Ajzen–Fishbein model of reasoned action and the health belief models. These models explain that individuals make rational decisions about behavior that affects their health when they are aware of associated health problems, have some knowledge about these problems and make a judgment about the level of risk involved in not changing their behavior. Thus, the willingness to change behavior is determined by perceptions and beliefs. In order to change, people must perceive that their current behavior is detrimental to their health and that taking action is likely to reduce the risk. Perceptions and beliefs are shaped by knowledge, which is acquired from exposure to information

sources along with personal efforts to obtain information.

Considerable information about consumer knowledge and self-reported food safety behavior is available, but observational studies suggest that large numbers of consumers follow unsafe food handling practices. Redmond and Griffith (2003) believe that observational studies provide a more realistic indication of food safety behavior in domestic food preparation because in their work, knowledge, attitudes and self-reported practices did not correspond to observed behavior.

Woodburn and Raab (1997) showed that respondents were not good at identifying either the foodborne illness or the groups of people particularly at risk for food poisoning. They also found that 40% of the 100 Oregon food preparers either believed that contaminated foods could not be made safe to eat or they did not know how to do so. After observing 108 consumers during all stages of the purchase, preparation, cooking and storage of one of four recipes, Worsfold and Griffith (1997) saw multiple examples of poor food handling practices leading to great potential for cross-contamination and subsequent food poisoning. On the other hand, a study found that food safety was rated as significantly more important when main meal planners were food shopping if they had one or more household members belonging to higher risk groups (Woodburn and Raab, 1997).

Awareness, knowledge and judgment can also be affected by habits and perceptions that result from social, cultural and economic influences. These may develop at an early age and become deeply ingrained. This can sometimes be due to stereotype behavior, where attitudes are developed without direct experience with the food in question (Cardello *et al.*, 1996). Lifestyle changes have also been shown to be influential in consumers' attitudes towards the safety of food handling. In April 1996, the American Meat Institute commissioned a study of 1000 adults in the US and concluded that lifestyle changes had had an impact on food behavior. These include an increasing number of women in the workforce, limited commitment to food preparation and a greater number

of single heads of households. Consumers appeared to be more interested in convenience and saving time than in proper food handling and preparation (American Meat Institute, 1996; Collins, 1997).

According to economic theory, the demand for food safety is determined by consumers' willingness to pay for additional safety, and it is assumed that they are willing to pay less for each successive unit of safety (i.e. increasing marginal costs but diminishing marginal benefits). The supply of safety is determined by the cost of producing safety by profit-seeking firms. The market for food safety will therefore be in equilibrium when the price that consumers are willing to pay for increases in safety is equal to the price at which suppliers are able to produce the increases. At such equilibrium, the level of safety supplied by the market will reflect a level of risk which is non-zero but acceptable. Extrapolating from this, it is suggested that consumer demand for food safety is increased when the gross production of a country (gross domestic product) is increased, since the average consumer is equipped with higher purchasing power (Tangermann, 1986). Familiarity with food safety hazards is likely to reduce the effectiveness of hazard warnings (Breakwell, 2000). If the consequences of a health hazard are more immediate, it is easier to visualize the risk; this, in turn, elevates the sense of danger.

2.3.2   Knowledge versus behavior

Raab and Woodburn (1997) point out that there is a disparity between food safety knowledge and self-reported practices. Knowledge, attitudes and behavior toward food safety have been shown to differ with demographic and socioeconomic factors. A large sample of senior undergraduate students in Greece completed a survey about food safety knowledge and practices (Lazou, 2012). Respondents answered less than 40% of questions about knowledge and behavior correctly. They were generally knowledgeable about the effects of freezing on bacteria and followed best practices for hand washing and prevention of cross-contamination. Knowledge of food safety

was similar for males and females, but there was a marked difference between the two genders when it came to food handling behavior, for which males scored considerably lower.

In a study of the knowledge and behavior of hamburger meat of 1439 consumers in Texas, McIntosh *et al.* (1994) concluded that while better-educated people tended to choose health and safety as their reasons for cooking preference, these respondents were more likely to prefer their hamburgers to be less well cooked. Thus, the reasons for cooking preferences may be not be influenced by either knowledge or mass media exposure. Furthermore, many individuals may not associate what they know about the risks of improperly cooked hamburger with their own practices.

In a telephone survey of 100 Oregon food preparers, Woodburn and Raab (1997) found that, even with a high awareness of foodborne illness, 20% of respondents reported unsafe practices in their food preparation. This is despite the fact that 56% of the respondents knew that they could thoroughly cook food contaminated with *Salmonella* to make it safe to consume and 59% knew this for *E. coli*.

The disparity between food safety knowledge and food handling practices of consumers can be attributed partly to optimistic bias, where people believe that they are less at risk from a hazard than other people (Miles *et al.*, 1999). Optimistic bias may be caused by the fact that most members of the public are rarely given personalized information about their vulnerability to a hazard; they get information about risk to the population in general and infer their own risk status. This may result in a noticeable discrepancy between people's perceived personal risk and their actual risk status (Frewer *et al.*, 1994).

Optimistic bias has been to blame for food poisoning from food prepared in the home and food prepared by others. Raab and Woodburn (1997) found that about one-quarter of respondents believed that food eaten at home had a lower risk of causing food poisoning than food eaten out. Frewer *et al.* (1994) also found that respondents considered that they had substantial control

over the risks; they perceived low personal risk and high knowledge about food poisoning in the home. Optimistic bias is important, in that it may hinder initiatives designed to promote risk-reducing behavior. People may ignore risk communications, assuming that these messages are aimed at more vulnerable individuals, or that they are in control of the potential hazards and know enough to deal with them effectively.

2.3.3 Influence of consumer demographics

Available literature has indicated that, overall, consumer attitudes towards food safety are influenced by demographic and socio-economic factors such as gender, age, educational level and economic status. A study of adults from the US Food and Drug Administration's 2006 Food Safety Survey (Anderson *et al.*, 2011) showed that adults 60 years of age and older were more likely to follow recommended food safety practices than those younger than 60 years. Among adults 60 years of age and older, there was a greater awareness of food safety risk among women, those with less education and non-white individuals. Another US study demonstrated that the food safety perceptions of elderly consumers who participated in congregate meal and home-delivered meal programs varied by geographic location, age, marital status and household composition; these demographic variables as well as gender, race and education influenced participants' self-reported food safety behaviors. Interestingly, there were significant differences in self-reported emergency food preparedness by race and level of education (Roseman, 2007).

Age, gender and level of education have been shown to affect food safety perceptions of ethnic restaurants. In a study of consumer food safety perceptions of Asian and Mexican restaurants in California and Florida, participants who were older, female and who had lower levels of education were most concerned about food safety. The majority of participants in this study reported that they had not suffered from foodborne illness, yet that they had experienced its classic signs (nausea, diarrhea and vomiting) (Lee *et al.*, 2012).

Pregnant women are one of the most vulnerable groups of consumers to foodborne diseases (McCabe-Sellers and Beattie, 2004), although there are few studies related to food safety attitudes, knowledge and behavior within this group. A study of 491 Slovenian women (291 pregnant and 200 non-pregnant) showed that all had a high level of awareness of food safety issues; this awareness varied significantly with the town of residence and age of respondent. Food safety practices differed between the two groups: for example, pregnant women more frequently thawed food at room temperature but paid more attention to 'best before' dates than did their non-pregnant counterparts (Jevšnik *et al.*, 2008).

The American multi-state survey conducted by Altekruse *et al.* (1999) reported that men were more likely to report risky practices than women. The survey results also indicated that the prevalence of most risky behaviors increased with increasing socio-economic status.

Burger (1998) interviewed 197 men and 94 women from a coastal population in New Jersey. He found that there were significant gender differences in the perceptions of the safety of fish, ducks and deer. Women generally believed that it was more risky to eat these foods than men. However, it was generally thought that it was safer to eat fish that consumers caught themselves or bought in a fish store than fish purchased from a supermarket.

2.3.4 Knowledge and behavior

In recent years, considerable resources have been devoted to increasing the level of food safety knowledge among consumers. For a food safety program to be effective, there must be a thorough understanding of the channels of communication most used by the target audience. Common media used in the past included television, radio, newspapers and magazines and publically distributed leaflets; the use of television as a source of information about food safety was shown to lead to a superior knowledge of foodborne disease whereas both television and print media were most effective in generating a willingness to change behavior

(McIntosh *et al.*, 1994). With the current popularity of social media, the optimal format for delivering food safety education may have changed. A single medium is not ideal; food safety education for pregnant women may be delivered effectively using social media, but what about the elderly who may depend on others for their food preparation? Men care less about food safety than women do (Aakkula *et al.*, 2005), so how are they best reached? The use of smartphone technology (with software such as the '4 Day Throw Away' application) is an innovative way to deliver food safety information to young families who might have questions about food safety (Albrecht *et al.*, 2012). Clearly, the choice of medium and the content of the message are both critical considerations (McCarthy and Brennan, 2009).

There is no doubt that consumers could benefit from food safety education, especially education about food-handling behaviors that are most likely to cause foodborne illness. The emphasis of such food safety education should be directly correlated to the relative frequency of behaviors that cause foodborne illnesses, i.e. more emphasis should be placed on behaviors that can be shown to cause more foodborne illness (Medeiros *et al.*, 2001). In the US, personal hygiene (especially hand-washing) and adequate cooking/avoiding cross-contamination should be given the greatest emphasis since these behaviors are reported to be associated with 10 million and 3.4 million cases of foodborne illness per year, respectively. Safe storage temperatures and avoiding food from unsafe sources should be given relatively less emphasis since these behaviors have been associated with comparatively fewer foodborne illnesses (0.5 million and 10,000 cases per year in the US, respectively) (Medeiros *et al.*, 2001). Over time, the emphasis in food safety education may change with the relative frequency of foodborne illnesses and consumer behaviors.

Consumer education messages should include the ubiquity of microorganisms, a comprehensive description of foodborne illnesses and prevention strategies. Product labels should contain food-handling information and warnings for special populations; information about foods processed by newer safety-enhancing technologies should be made more widely available. Knowledge of the consequences of unsafe practices can enhance motivation and adherence to safety guidelines. These are the responsibilities that should be shared by the health community, food industry, regulators and the media (Bruhn, 1997).

Food safety education and training should emphasize risk management and should target high-risk groups, as well as those preparing food for people in these groups. It must be updated regularly, and be based on emerging food safety trends if it is to remain effective in the long term.

2.4   Conclusion and outlook

Consumers' attitudes toward food safety, food handling practices and new technologies are of interest to a wide range of stakeholders such as food producers, processors and retailers and public health professionals. These attitudes are complex, and are formed by the interaction of factors such as demographics, philosophical perspectives and social, cultural and economic conditions.

Numerous technologies have been discussed in this chapter. Not surprisingly, consumers are likely to perceive personal and/or societal risks with each of these technologies. Those perceived risks vary with the nature of the technology; concerns related to technologies considered 'bioactive' may be escalated for ethical reasons and/or because of their unpredictable nature (Frewer *et al.*, 2011).

Furthermore, the balance of risks and benefits is important to consider. For example, do industry and consumers share the benefits equally or are the benefits disproportionately in favor of one stakeholder over the other? Do consumers have control over their exposure to a technology? In a review by Frewer *et al.* (2011), the least-controversial technologies were those that were limited in terms of their bioactive nature.

Finally, individual differences among consumers will have a profound influence on their acceptance of new food technologies and are likely to change over time. The challenge is to understand how to

best assist consumers in balancing the risks and benefits relative to their own frames of reference. There is a need to learn more about consumer attitudes and behaviors, create awareness of the benefits and risks of new technologies, promote trust in credible information sources and make available relevant food safety information.

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3

Educating for Food Safety

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Summary

Insufficient high-quality empirical evidence is available to make a determination about the effectiveness of food safety education on foodborne-disease-related outcomes. Food safety education training programs often do not approach transmission of knowledge in a systematic and theoretically sound fashion. They are often focused on knowledge outcomes judged by test scores and not behavior change outcomes, which are key to implementing safer food handling. Furthermore, food safety education needs to be provided in the native language of the learner in order to ensure better safety outcomes. Education is a crucial component, and is in need of improvement. By bringing the best scientific practices to food safety education, lives can be saved and suffering reduced. Developing more effective food safety education interventions is a goal worth pursuing.

3.1 Introduction

Worldwide, food safety is an important and pressing public health issue. The 2010 Global Burden of Disease (GBD) report, the largest ever systematic effort to describe the global distribution of suffering, ranks diarrheal disease as the fourth-most prevalent malady in the world after heart disease, pneumonia, and stroke (Murray *et al.*, 2013). The GBD estimate of diarrheal disease combines both waterborne and foodborne sources. Exact global statistics for foodborne disease do not exist as the vast majority of cases go undiagnosed or unreported, especially in resource-poor countries. Nonetheless, reported cases and outbreaks make clear that foodborne disease is a global problem.

To get a sense of the full picture, consider the United States (US) which is believed to have one of the safest food supplies in the world. However even within the US, with its sophisticated reporting mechanisms and world-class surveillance system hosted by the Centers for Disease

Control and Prevention (CDCP), an estimated 48 million Americans are sickened by foodborne disease each year resulting in 125,000 hospitalizations and 3000 deaths (Scallan *et al.*, 2011). On a global scale, this is just the tip of the iceberg. The very young (under age five) and the very poor, especially in Africa and Asia, are most at risk (Murray *et al.*, 2013). Between 1988 and 2007, 4093 outbreaks of foodborne disease were documented on every continent except Antarctica (Greig and Ravel, 2009). Of these, more than 2100 cases were documented in the US and another 1600 cases were documented in the European Union. According to this study, it would appear that foodborne disease is more of a problem for developed countries. However, the availability of data depends on reliable surveillance systems, which often do not exist in developing countries. It can also be assumed that countries known for being less transparent might under-report foodborne outbreaks. Outbreaks in some countries are therefore not reported as often, or at all. Using the GBD report as context, outbreaks in sub-Saharan Africa would be expected, for example. However, the only African countries included in Grieg and Ravel's study are South Africa, the richest country on the continent, and Zambia, a top 20 African GDP performer (CIA, 2013). Outbreaks in the Democratic Republic of Congo, for instance, may not have been reported for a variety of reasons such as lack of a strong central government and medical infrastructure and destabilization caused by ongoing fighting between the army and rebel militias. It is clear that foodborne disease affects all nations, but because of data availability and reliability the US and EU will be used as examples throughout this chapter.

To address the growing public health issue of foodborne disease, in May 2000 the 53rd World Health Assembly (the governing body of the World Health Organization or the WHO) adopted a resolution calling upon the WHO and its member states to recognize food safety as an essential public health function (WHO, 2002). The resolution also called for the development of food safety systems that enable a reduction in the burden of foodborne disease. The Food and Agriculture Organization (FAO), a specialized agency of the United Nations system, responded to this resolution by developing a framework for member states to plan, organize,, and implement a national food safety control system. The FAO recommends that all food safety control systems include the following components (FAO/WHO, 2003):

1. Food laws and regulations: adoption of relevant, science-based, enforceable food laws and regulations that are preventive rather than reactive and enforcement-oriented.
2. Food control management: coordination of policies and operations at the national level with clearly defined measures of accountability.
3. Inspection: administration and implementation of food laws and regulations that utilize a qualified, trained, efficient and honest food inspection service.
4. Food monitoring and epidemiological data: creation of adequate laboratory facilities for physical, microbiological, and chemical analyses of food samples run by qualified analysts who are using reliable methods.
5. Information, education, and communication: delivery of balanced, factual information, education, and communication to groups across the farm-to-table.

In this chapter the focus will be on the education component of the 5th FAO recommendation: information, education, and communication. Food safety information and communication are important specialties in their own right, and critical aspects of a successful food safety system. However, these two components are beyond the scope of this chapter.

Food safety education (also called food safety training) has been pursued globally as a way to decrease the burden of illness from foodborne disease, as demonstrated by the vast array of programs, easily searched online, to show that different groups across the farm-to-table continuum believe education is worth deploying. Governments, industry, colleges, and universities

have developed countless food safety education interventions. However, the concern is how effective are they, particularly at changing safe food handling practices? Only when proper practices are implemented will the number of cases of foodborne disease decrease.

Typically, the targeted outcome for most food safety interventions is improved knowledge about safe food handling practices, presumably because it is assumed that practices will improve as knowledge increases (but this might not always be the case). Having the appropriate knowledge, as well as positive attitudes, about an intended behavior are important prerequisites to taking action, but they do not guarantee action. *Implementation* of safe food handling practices is the action that actually reduces the burden of illness. Knowledge and attitudes alone are therefore not always enough to ensure implementation of safe food handling practices. Behavior change presumably requires more than a good presentation, a test, and a pledge to follow recommended safe food handling practices.

Critics correctly point out that many, if not most, education efforts to raise awareness of food safety are not approached in a systematic, scientific, and theoretically and pedagogically sound fashion. In fact, within the food safety field itself, some experts are skeptical about the effectiveness of education as a control strategy for foodborne disease. In the *IFT Expert Report on Emerging Microbiological Food Safety Issues: Implications for Control in the 21st Century* (IFT, 2002) the authors, a panel of highly accomplished experts, do acknowledge food safety education as a control strategy but are unconvinced of its effectiveness.

These authors' beliefs are also supported by the literature. In 2007, a systematic literature review was published in *Food Control*. The review summarizes the methods and results of studies that reported the effectiveness of food safety and food hygiene training in the commercial sector of the food industry (Egan *et al.*, 2007). The authors point out that there is scant empirical evidence that documents the effectiveness of educational interventions at improving safe food handling practices among food handlers. Most evidence collected was considered fair to poor by scientific standards, a significant argument from the IFT skeptics' point of view. Most evidence about the effectiveness of food safety education is derived from pilot studies with no control groups. This indicates that the scientific community is only just beginning to gather empirical evidence to prove the effectiveness of food safety interventions. Skeptics and proponents can both agree that until substantial high-quality empirical evidence has been collected and analyzed, a decisive judgment of the effectiveness of food safety education at reducing the burden of illness from foodborne disease cannot be made. This suggests more work needs to be done to either improve or prove food safety training and education.

3.2 Food safety education targeting food handlers

For the purposes of this chapter, food handlers are defined as farm workers, production facility workers, and restaurant workers. Like Egan *et al.* (2007), the authors of this chapter also conducted a systematic search of the English-language literature in 52 databases to identify studies in which food safety education programs or training targeting food handlers were evaluated (Table 3.1). Egan *et al.* (2007) located 46 studies between 1975 and 2004. The authors of this chapter located 19 studies dating from 1994 to 2012. The study settings were mostly in the United States, but articles from Canada, the UK, and Israel were also included. Interventions targeted a variety of food handlers including farm workers, production facility workers, restaurant workers, and institutional workers. Only three studies mentioned that the intervention was available in a language other than English, while two other studies made the assessment method available in other languages. These languages include Spanish, Chinese, Vietnamese, and several eastern European languages (Smith and Shillam, 2000; Anding *et al.*, 2007; Averett *et al.*, 2011; Ratnapradipa *et al.*, 2011; Dworkin *et al.*, 2012).

Table 3.1 Analysis of food safety education interventions (N = 19) published in the English language during 1994–2012. (Source: Shadish et al., 2002)

Commercial foodservice establishments

Authors	Sample size and setting	Study location	Intervention	Research design	Assessment methods	Measurement	Results
Kirby and Gardiner (1997)	20 foodservice establishments and 10 controls	England	Basic food handler hygiene training	Pre-test/post-test control group design (not randomized)	Audit	Observed behaviors	No significant difference found between scores on first and second visits.
Cotterchio et al. (1998)	94 restaurants: 40 control and 54 experimental	Massachusetts, USA	15-hour food manager training	Pre-test/post-test control group design (not randomized)	Inspections	Inspection scores	Significant improvement in inspection scores when managers attended training sustained over a 2-year follow-up period.
Raval-Nelson and Smith (1999)	50 certified and 50 non-certified foodservice workers	Pennsylvania, USA	ServSafe® certification program	Static group comparison (randomized)	Telephone survey	Knowledge	• ≥94% correctly answered wiping hands on apron and separate storage of cooked and uncooked meats. • Both groups had low scores for internal temperature of chicken.
Smith and Shillam (2000)	240 workers from 36 restaurants	Colorado, USA	10-minute videotape on basic food safety shown on site	One-group pre-test/post-test/post-test design (not randomized)	Exam in English, Spanish; or Chinese; inspections	Knowledge; inspection scores; violations	• Test scores increased significantly from 44.7% to 73.2%. • Inspection scores increased significantly from 74.1 to 81.9.
McElroy and Cutter (2004)	615 foodservice managers	Pennsylvania, USA	Statewide Food Safety Certification utilizing ServSafe®	One shot case study (not randomized)	Retrospective survey	Self-reported behaviors	• 86% of participants 'moderately likely' to practice food safety before training. • 93% 'very likely' to practice food safety after training.
Shanley et al. (2004)	50 foodservice workers: 22 in traditional group and 28 in distance group	Connecticut, USA	Traditional and distance education classes on sanitation and food safety	Factorial design (not randomized)	Course test; national exam	Knowledge	• Test scores increased from 45 to 75 for traditional group and 47 to 73 for distance group. • Scores on national exam similar for traditional (75%) and distance (73%) groups.

Reference	Population	Location	Intervention	Design	Instrument	Outcome	Results
Hammond *et al.* (2005)	Restaurants and catering companies	Florida, USA	Certification program for food workers	One-group pre-test/post-test design (not randomized)	Foodborne outbreak data	Number of foodborne disease outbreaks and cases	• Number of foodborne outbreaks decreased from 1001 to 581. • Number of foodborne disease cases decreased from 5651 to 3582.
Anding *et al.* (2007)	189 food managers	Texas, USA	'Food Safety: It's Our Business' Food Manager class	One shot case study (not randomized)	Retrospective survey in English or Spanish	Self-reported behaviors	Significant increase in all 12 food safety practices after the course.
Roberts *et al.* (2008)	402 employees from 31 restaurants	Kansas, Missouri and Iowa, USA	4-hour ServSafe® class	One-group pre-test/post-test design (not randomized)	Assessment; observation form	Knowledge; observed behaviors	• Knowledge scores increased significantly. • Percentage of correct behaviors increased significantly. • Post-training knowledge scores did not influence behaviors.
York *et al.* (2009)	368 restaurant employees	Kansas, Missouri and Iowa, USA	1. ServSafe® training 2. Intervention 3. Training and intervention 4. Control group	Factorial design (randomized)	Questionnaire; observation form	Behavioral barriers; observed compliance	• Training/intervention group perceived more control over barriers than intervention or training groups alone. • Compliance scores higher with combined training/intervention group.
Chapman *et al.* (2010)	47 food handlers from 8 food service facilities	Ontario, Canada	Food safety info sheets	One-group pre-test/post-test design (not randomized)	Video recordings	Observed behaviors	• Hand washing attempts and correct hand washing events increased significantly. • Indirect and direct cross-contamination events decreased significantly.
Averett *et al.* (2011)	Restaurants, retail stores and school cafeterias	Missouri, USA	2 hour lecture in English, Spanish, Chinese or Vietnamese	One-group pre-test/post-test design (not randomized)	Inspection	Food handler-related violations; control violations	Food handler-related and control violations decreased significantly (12.2% and 29%, respectively).
Ratnapradipa *et al.* (2011)	32 Eastern European restaurant workers	Utah, USA	Trained in native language by own children or in English by instructor	Pre-test/post-test control group design (not randomized)	Test	Knowledge	Participants trained by children showed significantly greater improvement in understanding of food handling compared to participants trained by instructor

(Continued)

Table 3.1 (Continued)

Authors	Sample size and setting	Study location	Intervention	Research design	Assessment methods	Measurement	Results
Dworkin et al. (2012)	229 food handlers: 128 in intervention group and 101 in control group	Illinois, USA	Chicago Educational Food Handler project in English or Spanish	Pre-test/post-test control group design (randomized)	Questionnaire	Knowledge	• Knowledge scores of those who read the intervention increased significantly from 67% to 73%. • Difference in knowledge score by type of materials read was not statistically significant.
Rebellato et al. (2012)	1042 food handlers	Ontario, Canada	PROTON self-study using program manual or lectured course	One-group pre-test/post-test design (not randomized)	Test	Knowledge; attitudes; self-reported behaviors	• Significant increase in knowledge from 63% to 76%. • Largest change in behavior in washing hands before using gloves and wearing headgear when handling food (12.6% increase each).

Other (institutions, processing facilities, and farms)

Authors	Sample size and setting	Study location	Intervention	Research design	Assessment methods	Measurement	Results
Soneff et al. (1994)	46 small adult-care facilities	British Columbia, Canada	Content presented by registered dietician or in a manual	Factorial design (randomized)	Facility audit	Observed behaviors	Facilities that used workshop and manual had significantly greater improvement than manual only facilities.
Cohen et al. (2001)	774 food samples in a food processing facility	Israel	Program based on the guidelines of Good Manufacturing Practices	One-group pre-test/post-test design (not randomized)	Microbial samples of food	Total count of bacteria, mold and yeast	• Samples classified as high quality increased from 76.1% to 87%. • Food quality improved significantly in 3 preparation departments and main kitchen.
Staskel (2006)	Food service managers, head cooks and directors from 32 child care centers	Texas, USA	National Food Service Management Institute 'Serving It Safe' class	One-group pre-test/post-test design (not randomized)	Food Safety Assessment form; swabs of various surfaces	Observed behaviors; compliance with food safety standards; microbial quality	• Participant attendance of training did not significantly affect change in FSA scores. • No significant differences seen in number, type or locations of bacteria samples based on training.
Soon and Baines (2012)	62 fresh produce farm workers from 6 farms	United Kingdom	Food safety course: booklet, slides, demonstrations	One-group pre-test/post-test design (not randomized)	Questionnaire	Knowledge; behavior intention	• Significant improvement in participant knowledge in five of eight variables. • Participants more likely to wash hands when perceived fewer barriers to washing hands.

Eight studies measured change in knowledge using a variety of research designs including a one-group pre-test/post-test design ($n=4$), a pre-test/post-test control group design ($n=2$), a static group comparison ($n=1$), and a factorial design ($n=1$) (Raval-Nelson and Smith, 1999; Smith and Shillam, 2000; Shanley *et al.*, 2004; Roberts *et al.*, 2008; Ratnapradipa *et al.*, 2011; Dworkin *et al.*, 2012; Rebellato *et al.*, 2012; Soon and Baines 2012). Of these research designs, the pre-test/post-test control group design yields the highest-quality evidence if study subjects are randomized (Shadish *et al.*, 2002). Four studies also assessed other factors including inspection scores, self-reported behaviors, behavioral intentions, and observed behaviors (Smith and Shillam 2000; Roberts *et al.*, 2008; Rebellato *et al.*, 2012; Soon and Baines 2012).

To determine behavior change, four studies used participants' self-reported changes in practices or intention to change behaviors as a surrogate for actual practices (McElroy and Cutter 2004; Anding *et al.*, 2007; Rebellato *et al.*, 2012; Soon and Baines, 2012). This approach might be biased yielding unreliable results however, because participants may report that they would adopt a practice because it is socially acceptable or desirable and not necessarily because they *will* adopt it. Furthermore, it is well known that self-reported results are inherently biased; caution must therefore be exercised when making predictions using this type of data (Donaldson and Grant-Vallone, 2002). Presumably better data would come from studies in which food handlers' actual practices were observed.

Of the literature identified, the authors of six articles observed behaviors (Soneff *et al.*, 1994; Kirby and Gardiner, 1997; Staskel 2006; Roberts *et al.*, 2008; York *et al.*, 2009; Chapman *et al.*, 2010). Most of these studies (5/6) observed food handler behaviors on site by having a trained data collector complete a checklist or take notes. Chapman *et al.* (2010) installed video cameras to record behaviors and watched behavioral events with the benefit of stop/pause/rewind actions. While these direct observation studies appeared to be the most successful at documenting food safety

practices, they too are biased because the food workers knew they were being observed and may have been on 'best behavior' while under the watchful gaze of the observers or the cameras.

In order to remove as much bias as possible, the authors of six studies used other indicators to determine if an intervention was successful at improving safe food handling practices (Cotterchio *et al.* 1998; Smith and Shillam, 2000; Cohen *et al.*, 2001; Hammond *et al.*, 2005; Staskel, 2006; Averett *et al.*, 2011). These indicators included inspection scores, foodborne outbreak data, and the microbiological quality of foods and surfaces. The strength of the association between these types of indicators and specific changes in safe food handling practices was not clear. However, this approach is promising and should be explored further.

Specific results of the food safety interventions listed in Table 3.1 also vary. All published papers measuring change in knowledge reported a significant increase in overall knowledge scores after participants were exposed to the food safety intervention (1.3–39.5% increase). However, scores after the intervention were still low for most of the studies with post-test scores averaging about 73% (44.1–97%). Those studies measuring indicators other than knowledge, attitudes, and practices reported a significant improvement after the intervention. Three studies compared inspection scores; all reported increased scores after either managers or food handlers were exposed to the intervention (7–29% increase) (Cotterchio *et al.*, 1998; Smith and Shillam, 2000; Averett *et al.*, 2011). The authors of two studies reported that inspection scores continued to increase one or two years after the intervention but not significantly (Cotterchio *et al.*, 1998; Smith and Shillam 2000). Hammond *et al.* (2005) compared the number of foodborne outbreaks before and after implementation of mandatory food safety training for all foodservice workers in Florida, USA. Their results showed that the overall occurrence of outbreaks decreased from 250 per year to 194 per year after implementation of mandatory training. Of those studies in which the microbio-

logical quality of foods or surfaces within facilities were evaluated, only Cohen *et al.* (2001) showed an increase in sanitation. In their study, the percentage of food samples classified as 'high quality' increased from 76.1% before training to 87.0% after the training. On the other hand, Staskel (2006) found no significant difference in the number, types, or locations of bacterial samples after workers were trained.

In the four studies in which self-reported behaviors were used as an indicator of effectiveness, a significant improvement of participants' behaviors was reported (McElroy and Cutter, 2004; Anding *et al.*, 2007; Soon and Baines, 2012; ebellato *et al.*, 2012). On the other hand, the results varied widely in studies in which behaviors were directly observed. Significant improvements in food safety practices were reported in four studies (Soneff *et al.*, 1994; Roberts *et al.*, 2008; York *et al.*, 2009; Chapman *et al.*, 2010) while two showed no significant change in behaviors after the intervention (Kirby and Gardiner 1997; Staskel, 2006). As has been shown, the effectiveness of food safety education interventions vary widely and the authors' review of the literature concur with the findings of Egan *et al.* (2007).

3.3   Effective food safety education interventions

It could be concluded from the discussion of the literature in the previous section that we should abandon food safety education. That is not the assertion of the authors of this chapter, however. As stated earlier, the current literature suggests more work needs to be done to either improve or provide food safety training and education. We assert that food safety education can be an effective control strategy to reduce the burden of foodborne disease if approached in a *systematic manner* that addresses intervention design, instructional strategies, and learner assessment. A discussion of how to implement this approach is provided in the following sections.

3.3.1   Intervention design

Improved safe food handling practices should be the targeted outcome of an effective food safety education intervention. To achieve this, careful attention must be paid to the intervention design. To maximize its potential to be effective, an educational intervention must have as its underpinning an appropriate theoretical framework. This theoretical base provides structure and purposefulness to what and how content is presented. The theoretical framework must be grounded in an appropriate behavior theory and learning theory, as well as adult learning theory.

Many behavioral theories described in the literature are frequently used in health education and nutrition education interventions, but are infrequently used for food safety education. This is particularly true for those interventions that target food handlers working in commercial and institutional facilities. Table 3.2 presents a sample of behavior change theories that can be applied to food safety education. Further investigation was conducted to determine the implementation, practice, and evaluation of these theories in existing food safety curricula. After comparing the implementation of these theories within the context of food safety education, applicable theories were selected. There are several studies that stand out for their acknowledgement of behavior theory application and design. For example, Phillip Seaman proposes a new framework (not included in Table 3.2) called The Food Hygiene Training Model (Seaman, 2010). The model 'utilizes various theoretical models and educational theories to recognize the various influences on the training, beliefs, motivations, and conditions required for food handlers to perform safe food handling practices in the workplace'. Two studies of food safety interventions have applied behavior-change theories, such as the Health Belief Model (Schafer *et al.*, 1993; McArthur *et al.*, 2006), while another has used the theory of planned behavior and the theory of reasoned action (Lobb *et al.*, 2007).

Table 3.2 Behavior change theories

Behavior theories	Citation	Description	Applied research
Health Belief Model	Rosenstock *et al.* (1988)	A person's health-related behavior depends on the person's perception of four areas: the severity of the potential illness, the person's susceptibility to that illness, the benefits of taking a preventive action and the barriers to taking that action.	Schafer *et al.* (1993); McArthur *et al.* (2006)
Theory of Reasoned Action	Fishbein and Ajzen (1981)	Individual performance of a given behavior is primarily determined by a person's intention to perform that behavior.	Lobb *et al.* (2007)
Theory of Planned Behavior	Ajzen (1991)	TPB is the theory of reasoned action, but includes perceived behavioral control as an additional determinant of intentions and behavior.	
Social Learning/ Social Cognitive Theory	Bandura (1986)	Environmental factors represent situational influences and the environment in which the behavior is performed while personal factors include instincts, drives, traits and other individual motivational forces.	

Brief descriptions of selected behavior change theories follows (Table 3.2). We begin with the Health Belief Model which was developed in the 1950s by the US Public Health Service (Rosenstock *et al.*, 1988). This model is useful for determining a person's health thoughts. The Theory of Reasoned Action, Fishbein and Ajzen, suggests that a person who intends to perform a behavior is likely to complete the behavior (Fishbein and Ajzen, 1981). Later this theory was revised to include the Theory of Planned Behavior, which acknowledges perceived behavioral control as a determinant of intentions and behavior. Finally, Albert Bandura rounds out the behaviorist psychologists. Bandura's Social Learning Theory and Social Cognitive Theory acknowledge that environmental factors influence behavior, along with intuition, drive, and other motivational forces (Bandura 1986). Bandura is often cited in the context of his work in self-efficacy: the belief one has in oneself to accomplish something.

A sample of learning theories is presented next (Table 3.3). Bandura is mentioned again, as his work crosses over between behavior and learning.

Bandura's Theory of Observational Learning states that one learns by mimicking a model (Bandura, 2006). David Kolb popularized the Learning Style Inventory, which categorizes four tyles: converger (active experimentation–abstract conceptualization), accommodator (active experimentation–concrete experience), assimilator (reflective observation–abstract conceptualization), and diverger (reflective observation–concrete experience) (Kolb, 1984). Jack Mezirow's Transformative Learning Theory outlines the processes of learning and its implications for educators of adults. Transformative learning induces far-reaching change in the learner, producing a significant impact or paradigm shift which affect the learner's subsequent experiences (Mezirow, 2000). Finally, Lev Vygotskiĭ is the originator of Social Constructivism, a sociological theory of knowledge that claims that learning and development is a social, collaborative activity (Vygotskiĭ and Cole, 1978). One food safety study was found to have included in its design Transformative Learning Theory (Ellis, 2007). These theories are presented in an attempt to provide a starting

Table 3.3 Learning theories

Learning theories	Citation	Description	Applied research
Observational Learning Theory	Bandura (2006)	Learning by observing a model and then duplicating a skill, process, strategy, or task that is demonstrated by the model.	
Learning Styles Theory	Kolb (1984)	Four processes must be present for learning to occur: diverging, assimilating, converging, accommodating.	
Transformative Learning Theory	Mezirow (2000)	A theory of deep learning, transformative learning expands the consciousness through transformation of world view and capacities of the self.	Ellis (2007)
Social Constructivism	Vygotskiĭ and Cole (1978)	Learning and development is a social, collaborative activity	

point for food safety curriculum developers to begin their intervention design.

Food safety interventions targeting food handlers should also be grounded in adult learning theory as it is assumed that most participants of these programs will be adults. We therefore need to understand how adults learn best (Lieb, 1991). Andragogy (adult learning) is a theory that establishes set of assumptions about how adults learn. It suggests that learning should be problem-based and collaborative rather than didactic, and also emphasizes more equality between the teacher and learner (Knowles, 1972). Knowles identified six principles of adult learners:

- adults are internally motivated and self-directed;
- adults bring life experiences and knowledge to learning experiences;
- adults are goal oriented;
- adults are relevancy oriented;
- adults are practical; and
- adult learners like to be respected.

Additionally, Barak Rosenshine (Cicchinelli *et al.*, 2006; Rosenshine, 2012) has identified best-practice instructional strategies that are most likely to improve learner achievement across all content areas and across all grade levels.

Examples of learning touchstones include beginning a lesson with a short review of previous learning, limiting the amount of material students receive at one time, providing many examples, and re-teaching material when necessary. While the application of these principles is within the kindergarten to Grade 12 setting, these principles can easily be applied to interventions targeting adult learners.

In addition to being aware of the appropriate theoretical underpinning of an intervention, the content presented in an intervention must be science-based *and* practical. The term 'science-based' means the content should be up-to-date and supported by relevant research. Scientific evidence is derived from studies that (a) carefully identify and control for variables and (b) demonstrate the level of confidence with which outcomes and results can be associated with those variables. Another useful definition is offered by The National Academy of Sciences (2008): 'The use of evidence to construct testable explanations and predictions of natural phenomena, as well as the knowledge generated through this process.'

The US Food Code is an example of a science-based set of food safety regulations, as most of the regulatory provisions are based on published studies. Other science-based food codes include the European Food Law Code, the Food Law

Code of Practice (England), the Food Safety Authority of Ireland, the Australia New Zealand Food Standards Code, and the regulations of the Canadian Food Inspection Agency.

In the US many food safety education interventions are based on the regulatory provisions outlined in the US Food Code, reviewed every two years through the Conference for Food Protection. However, solely basing curricula on government regulations is a flawed approach because while the regulations are science-based they might not be based on the most current science. For example, the scientific basis upon which the US Food Code provisions relate to hand hygiene are clearly limited. A tremendous amount of information has been learned in the last 20 years, which could better help our understanding of hand hygiene (Fraser *et al.*, 2012).

In addition, implementation of science-based practices might not be practical under real-world conditions. Again, hand hygiene will be used as an example. It is widely reported in the literature that recommendations for hand hygiene are often not followed, even although millions of dollars have gone into funding initiatives to improve compliance (Fraser *et al.*, 2012). One underlying reason is that current recommendations are impractical under real-world food handling conditions. A second example is based on the prevention and control of human noroviruses in foodservice settings. Noroviruses have been identified as the number one cause of foodborne disease in the US and are believed to be the number one cause of acute gastrointestinal illness worldwide. One important control strategy for noroviruses is the clean-up of vomit and fecal matter. In the new Food Code in 2–501.11 it states:

> 'A FOOD ESTABLISHMENT shall have procedures for EMPLOYEES to follow when responding to vomiting or diarrheal events that involve the discharge of vomitus or fecal matter onto surfaces in the FOOD ESTABLISHMENT. The procedures shall address the specific actions EMPLOYEES must take to minimize the spread of contamination and the exposure of EMPLOYEES, consumers, FOOD, and surfaces to vomitus or fecal matter.' (US Department of Health and Human Services, 2009).

However, the challenge to this is that at present there are no universally accepted guidelines for the clean-up of vomit, so how this content is presented will vary widely until science-based, practical vomit clean-up methods are established. It is therefore not enough to develop a science-based curriculum. An effective curriculum must be based on content that is science-based and practical to implement.

3.3.2   Instructional strategies

A theoretically sound curriculum that is science-based and practical must then be delivered using proper instructional strategies. The two instructional strategies that will be discussed in this chapter are delivery method and the competence and credibility of the educator. Delivery methods can be characterized by their degree of formality and their degree of interaction. Traditionally, food safety education has been instructor-led in a classroom setting. In recent years however, e-learning methods have gained wide acceptance as a time-efficient training method (Phillips *et al.*, 2012).

Online training is heralded for its convenience and low cost, but offers fewer opportunities to receive clarification from an in-person instructor (Table 3.4). Proponents of face-to-face instruction often point to the active nature of learning in a classroom discussion, as opposed to passively clicking through a tutorial. However, the body of literature that compares online education to face-to-face is vast and growing, which is why we have limited our discussion as it is beyond the scope of this chapter. In addition, two other methods for teaching safe food handling principles must also be recognized: self-training and peer training. However, there is limited evidence available in the literature that describes the effectiveness of these methods.

In addition to the delivery method, the competence and credibility of the instructor must also be addressed. In the kindergarten through Grade

Table 3.4   Advantages and disadvantages of online versus face-to-face training

Training method	Advantages	Disadvantages
Online	Allows for learning in distant and disadvantaged locations; accessible from a standard Internet connection; fast transfer of information	Can isolate students from one another as well as the instructor.
Face-to-face	Promotes open exchange of ideas between students and with the instructor	Encourages passive learning; ignores individual learning differences between students; difficult to isolate and address learning deficiencies; others may dominate discussions so quieter personalities limited in their communication options for exchanging ideas and information

12 literature, teacher competence is correlated to student success (Goddard *et al.*, 2000). However, there is scant evidence to correlate the competence of the food safety educator to student success as it relates to changing safe food handling practices. Published literature from consumer behavior, social psychology, and related disciplines suggests that a highly credible source is more likely to lead to increased behavioral compliance than a low-credibility source (Maddux and Rogers, 1980; Sidney and Shuv-Ami, 1986). For example, Jones *et al.* (2006) found that participants receiving a positively framed communication from a credible source reported more positive exercise intentions and behaviors than participants who received negatively framed information from a non-credible source. Other studies have also shown that the degree of perceived credibility of the source influenced a recipient's intention to use suggestions made by the source (Bannister, 1986).

The effect of source credibility on persuasion indicates the superiority of a high-credibility source over a low-credibility source. Two important dimensions of source credibility are (1) expertise: the extent to which the communicator is perceived to be capable of making correct assertions; and (2) trustworthiness: the degree to which an audience perceives the communicator's information to be valid. Other dimensions include competence, dynamism, objectivity,

authoritativeness, and character (McCroskey, 1966; Berlo *et al.*, 1969). Petty and Cacioppo (1996) predict that strong arguments can produce major shifts in attitude in the desired direction of the communicator. A strong argument is one that generates favorable thoughts when it is heard and scrutinized. A weak argument can cause a boomerang effect that will last over time, defy other efforts to change it, and affect subsequent behavior.

A food safety educator who can present strong arguments about the importance of safe food handling and who is perceived to be a highly credible source of information by the target audience is believed to be more persuasive in promoting the adoption of practices to prevent foodborne illness. If a food safety educator does not have a comprehensive scientific understanding of the basic principles of food safety, the educator will not be able to adequately explain concepts or answer questions. The quality, effectiveness, and credibility of their efforts could therefore be inadequate. To ensure that credible food safety messages are delivered, food safety educators need more in-depth, up-to-date food safety training addressing current issues and trends relative to the scientific knowledge they need to function as highly effective educators.

Efforts have been made to develop competencies for US food industry regulators but not for food safety educators. The process and criteria

for a federal regulator to demonstrate proficiency in the required performance areas are described in the US FDA Procedures for Standardization and Certification of Retail Food Inspection/Training Officers (US Public Health Service, 2010). A few comprehensive food safety training opportunities exist for food safety regulators, such as the US FDA course Food Code, FD1012. This course focuses on training regulators about the Food Code and the corresponding public health rationale in order to prepare them to apply the Code during regulatory inspections. These courses are informative, but their greatest limitation is that they are designed for regulators. Most food safety educators are not regulators, so these programs are not appropriate for them or may not even be made available to them. All food safety educators, regardless of whether or not they are regulators, are in need of training to help them better understand the science behind basic food safety principles.

3.3.3 Learner assessment

Food safety education targeting food handlers varies in quality, implementation, and outcome. Effective interventions need to have a theoretical underpinning, be grounded in science-based content that is practical, and be delivered by competent, credible educators. If all of these tenants have been adhered to, the final, crucial piece: is how to assess learning?

A full-scale evaluation of a food safety education intervention is very difficult as it is resource intense and cannot be performed frequently, which is a limitation that must be recognized. As a result, most data used to determine the effect of an educational intervention are collected in a non-controlled setting with instruments that have not been tested for validity or reliability, hence the results are questionable. For example, most educators determine the effectiveness of a program by assessing the following: number of participants who attend a program, participants' reactions to the program, number of participants who successfully complete the certification examination, and food safety knowledge scores. Using

any of these independently or in combination as a measure of program impact is very limited.

A valid and reliable learner assessment method must be the centerpiece to proving that food safety education is effective. The best measure of effect would appear to be the adoption of safe food handling practices because it is assumed that foodborne illness is nearly 100% preventable if food is handled safely. Critics of food safety education can understandably point to this as a reason for their skepticism of the benefits of food safety education. Why then do educators so rarely measure the effects of their educational efforts on behavior change?

One reason may be that this falls outside the purview of the educator's assignment; for many educators, the goal for their students is a passing food safety knowledge test. Another reason may be that educators simply do not know how to reliably measure adoption of safe food handling practices. Further complicating matters, when educators do try to measure the effectiveness of their training program, they may not use a sound measurement instrument. Mederios *et al.* (2001) evaluated 12 food safety education curricula for consumers (not food handlers). One component of the review was to assess instrument reliability and/or validity. Only half of the evaluation instruments reviewed had been tested for reliability and/or validity and even then to varying extents. One conclusion was that more rigor was needed in the development and pretesting of assessment instruments if educators are to have confidence in the outcomes they report.

Measuring the adoption of safe food handling practices after a food safety education intervention is difficult; evaluation tools to reliably measure food handling practices are currently not available, and methods for collecting post-training data are often not appropriate or result in low response rates. Attention must be given to the development of valid, reliable, and easy-to-administer instruments that measure behavior change. Only when this type of instrument is available will food safety educators begin to be able to prove the value of food safety education.

3.3.4 Training in languages other than English

Language difficulties are perceived to be another major barrier to the adoption of safe food handling practices. The United States, a nation of immigrants, is a good case study for the importance of multi-language education. In the US, most food safety trainings are conducted in English which is not necessarily the first language of the trainee. In 2010, the foodservice industry employed more than two million immigrants, representing slightly more than 20% of US foodservice labor (Camarota, 2012). This is an estimate; the actual number could be even higher. Regardless of a nation's immigration policy, which is not the purpose of this chapter, language and cultural differences must be recognized as important factors when educating food handlers. Food safety educators must be pragmatic and proactive when addressing the needs of food handlers who are not fluent in the predominant language of the country in which they work. This applies everywhere, whether discussing Hispanics/Latinos in the United States, Moroccans in Spain, Indians in Saudi Arabia, or Chinese in Australia.

Using the US as an example, English and Spanish are the two most commonly spoken languages so much attention has been given to providing bilingual information. That is a good start, but there are 364 other spoken languages in the United States (Lewis, 2009). Given this fact, it is highly likely that food safety resources are needed in more than just English and Spanish.

The same can be said of other countries with diverse populations. India, for instance, has several hundred dialects and several lingua franca, Hindi, English, and Bengali among them.

In the US, a typical training format is to provide training materials in English, teach the class in English, then administer the certification exam in English, Spanish, or Chinese. There are three American National Standards Institute (ANSI) recognized food handler certifiers: National Registry of Food Service Professionals (NRFSP), Prometric, and the National Restaurant Association

(NRA) ServSafe® program. NRFSP offers exams in English, Spanish, and Chinese. Prometric accommodates English, Spanish, Chinese, Korean, and Vietnamese, and the NRA ServSafe® exam is available in English, Spanish, Chinese, Korean, Japanese, and French Canadian. The ServSafe® exam policy also allows for language accommodation through the use of an approved translator and a native language to English dictionary, both of which need to be provided by the test taker. These are admirable accommodations, but put the burden of finding and compensating a translator on the test-taker. Considering that the national average compensation for food handlers in the US is $10.30 an hour (Bureau of Labor Statistics, 2011), providing one's own translator may be out of reach for many food handlers.

The traditional delivery format therefore puts limited and non-English speakers at a disadvantage as they are often in a position of receiving information in one language and translating it into their own. Context and detail can be lost in translation. Because foodservice workers must understand what is being taught, presenting information in these workers' native languages would seem to be a logical practice. Recognizing the first language of food handlers and offering food safety training in those languages will presumably lead to more successful food safety outcomes.

Hoffman and Taylor (2005) confirmed this, reporting that delivering food safety information in the language that a person understands best can improve productivity, compliance, and morale, which are all critical to the adoption of safe food handling practices. In the US, food safety education materials are widely available in English, Spanish, and, to a lesser extent, Chinese. To adequately meet the needs of the foodservice industry, food safety professionals must give more attention to smaller language groups working within the industry. A search for existing materials showed that materials are available in many languages. However, it is not known how these materials have been reviewed for technical and language accuracy. Also, many are not free. To date, no comprehensive set of food safety training materials are available in all the world's

major languages. Safe food handling practices, proven by science, should be culture-blind. In other words, safe is safe, despite custom or tradition. It is therefore conceivable that safe food handling practices could be offered online, for free, in all the world's major languages. To begin achieving that goal, the authors have created a website (http://www.foodsafetysite.com) that addresses food safety training that is up to code in the United States, can be used in any country, and which offers training materials in English, Spanish, Arabic, Simplified Chinese, and Russian. At the moment, a wide variety of high-quality Chinese language materials (Mandarin and Cantonese) based on US food service standards are available at a sister site (http://chinesefood-safety.com).

Food safety materials are often developed on demand. In an audience that has several first languages among its members, it is more likely that materials will be developed for the majority. For example, a large restaurant group has workers who speak Spanish, Portuguese, English, and Mandarin; however, 80% of the food handlers speak Spanish as their first language. The training materials are therefore presented in English and Spanish, to the detriment of the Portuguese and Mandarin speakers. To provide limited- or non-English-speaking foodservice workers with opportunities for success, comprehensive sets of food safety training resources based on a high-quality food code – be it the FDA Food Code, the European Food Law Code, The Food Law Code of Practice (England), the Food Safety Authority of Ireland, the Australia New Zealand Food Standards Code, Canadian Food Inspection Agency, or similar national food safety standards – must be broadly available in a wider array of languages at a minimal cost. A review of these international food code materials available in multiple-languages reveals translations of some, but crucially not *all*, materials into the following languages: Arabic, Chinese, Czech, Dutch, French, German, Hindi, Italian, Japanese, Korean, Latvian, Polish, Portuguese, Romanian, Russian, Spanish, and Thai. The availability of translated materials is highly variable. A bright spot on the horizon is that translation software is always improving; in the near future we may see this problem addressed in an affordable and comprehensive way as countries with high immigrant populations, such the US, Canada, Australia, and EU members, find ways to deploy accurate translations of their food safety training materials in their constituents' first languages.

3.4 Future outlook

Insufficient high-quality empirical evidence is available to make a determination about the effectiveness of food safety education on food-borne-disease-related outcomes. Food safety education training programs often do not approach transmission of knowledge in a systematic or theoretically sound fashion. They are often focused on knowledge outcomes, judged by test scores and not behavior change outcomes, which are key to implementing safer food handling. Furthermore, there are varying standards for food safety from country to country. Food safety education needs to be provided in the native tongue of the learner in order to ensure better safety outcomes. As developing countries become richer and the growing middle classes begin to eat food prepared outside their own homes, particular attention must be paid to those who are working in restaurants around the world. Foodborne disease, especially those caused by noroviruses, is preventable with the right combination of technology, education, and practice. Education is a crucial component, and is in need of improvement. By bringing the best scientific practices to food safety education, lives can be saved and suffering reduced. Developing more effective food safety education interventions is a goal worth pursuing.

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4

Food Safety Training in Food Services

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Summary

This chapter presents recommendations concerning the training of food handlers in food services, with special focus on food safety. First the chapter addresses legal issues. Further, issues involved in the evaluation of training programs are described. Finally, we discuss important steps in the planning of training programs, the themes to be addressed, the length, and the style of language used. The information found in this chapter can be used to develop effective training programs that reinforce the technical capacity of food handlers and help to guarantee food safety in food services.

4.1 Introduction

Outbreaks of foodborne illnesses or diseases are a major public health problem (WHO, 2006). In 2009 and 2010 in the United States for example, 1527 foodborne disease outbreaks were reported, affecting 29,444 people and leading to 1184 hospitalizations and 23 deaths. Of these outbreaks, 766 were due to the consumption of food at a single place, and 48% of the outbreaks the food was consumed in restaurants or delis (CDC, 2013).

Most of the occurrences of foodborne illness can be prevented by proper food handling (WHO, 2006). After an investigation of outbreaks at restaurants that were reported to the Centers for Disease Control and Prevention (CDC) from 1982 to 1997, Hedberg *et al.* (2008) concluded that

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of 906 outbreaks, 33% were associated with poor personal hygiene among employees. The others were related to improper food preparation practices (e.g. improper cooking and warming), and poor cleaning of equipment. Upon investigating the risk factors for foodborne illnesses in full service restaurants, the US Food and Drug Administration (FDA, 2009) noted that the inability to control timing and temperature in food preparation was the most frequent factor, followed by poor personal hygiene among food handlers and contamination of equipment.

The realization of employee training can be an important strategy for preventing or decreasing the risks of food contamination, adapting the practices of the handlers, and promoting food safety in food services (Mitchell *et al.*, 2007; Medeiros *et al.*, 2011). Effective training programs must therefore be developed (WHO, 2002) which can be conducted by government or non-governmental entities (WHO, 2000). It is observed, however, that the training of food handlers in food services is mandatory according to food service regulations in many countries, states, and municipalities (FAO/WHO, 2005).

To obtain good results with the training programs, a need was identified for proper planning and evaluation of the activities (WHO, 2000). This chapter therefore presents important information that must be considered in the training of food handlers in food services, based on an analysis of the literature. We first look at the training of food service employees in the context of government regulations, before presenting recommendations concerning the evaluation and planning of training programs.

4.2   Legislation about training

Seeking to guarantee food safety, different countries establish norms for the activities of food handlers in food services. The topics addressed in the regulations concerning employees include: the need for employee training; factors related to their health conditions, including information

about contagious illnesses and the presence of wounds; clarification about personal hygiene and hand washing; information about the use of gloves; the need to supervise activities; and others (e.g. Mercosul, 1996; Brasil, 2004; European Union, 2004; FDA, 2011). In general, employee training in food services is related to protecting public health, and to the safety and integrity of the food offered (Mercosul, 1996; European Union, 2004; FDA, 2011). The regulations for this purpose frequently address the issues to be discussed in training, documentation attesting that the training took place, and the inspection of this training (Martin, 2003; Brasil, 2004; FDA, 2011). Listed in the following sections are some issues that should be included in training, according to the regulations in selected locations.

4.2.1   European Union

According to European laws, food handlers must be trained about hygiene issues, in accordance with the needs of their professional activity, and should be given guidance about the correct application of the principles of Hazard Analysis and Critical Control Points (HACCP). In addition, the countries must attend all the requirements of the national laws related to the training of food sector personnel (European Union, 2004).

4.2.2   United States

The topics recommended to be included in the training of food handlers and/or managers of establishments in the United States include: the risk of food contamination (e.g. laws in the states of Virginia, Oregon, New Jersey, Minnesota, Kansas, Delaware, Pennsylvania, and others); hand washing procedures, indicating when and how to wash (e.g. laws in the states of Virginia, Oregon, New Jersey, Kansas, Delaware, Pennsylvania, and others); issues of personal hygiene, such as nail care (e.g. laws in the states of Virginia, Oregon, New Jersey, Delaware, Pennsylvania, and others); prohibition of the use of ornaments, such as jewelry (e.g. laws in the states of Virginia, Oregon, New Jersey, Delaware, and others); good handling and hygiene

practices (e.g. laws in the states of Virginia, Oregon, Delaware, Louisiana, Pennsylvania, and others); food allergies (e.g. laws in the state of Oregon); HACCP (e.g. laws in the states of Oregon and New Jersey), and others (FDA, 2011).

In addition to the previously mentioned issues, in some states laws mention the importance of training employees in food safety, according to the demands of the function they exercise in the institution (e.g. laws in the states of Virginia, Vermont, Rhode Island, Oregon, New Jersey, Kansas, Indiana, Idaho, Delaware, Arizona, Maine, Maryland, Pennsylvania, Utah, and others) (FDA, 2011).

4.2.3   Mercosur

For the Common Market of the South (Mercosur), regulations establish that food service operators receive proper and continuous instruction in issues of hygiene, food handling, personal hygiene, and hygienic-sanitary measures to avoid food contaminations (Mercosur, 1996).

4.2.4   Brazil

Brazilian legislation concerning food services establishes that the issues that must be addressed in food handler training are: food contaminants; foodborne diseases; hygienic food handling; and good handling practices.

The need to document employee training, with a focus on food safety, is also highlighted in some laws as in some states of the United States (e.g. Virginia, Oregon, New Jersey, and others), in Brazil (Brasil, 2004), Argentina (Argentina, 1969), and other locations. A document attesting that these activities have taken place must be present at the food services establishments and available whenever necessary, such as during inspections (Brasil, 2004; FDA, 2011).

Another issue addressed in the laws is employee supervision, which can be conducted by the managers of establishments. These professionals must have sufficient knowledge to supervise the other workers, and their training must be accredited by the inspection agencies (e.g. Brasil, 2004; European Union, 2004; FDA, 2011). Some laws establish additional measures related to the training of food handlers, such as the number of hours of training or the materials to be used in them.

4.3   Evaluation of the programs

Evaluation of the training programs is essential for identifying and analyzing their results. There are various forms of classifying and denominating the evaluations of the programs. In the context of this chapter, it is considered important to present an overview of the evaluation and monitoring of the process and evaluation of the impact of the programs.

The impact evaluation assesses whether the program produces the intended effects (Rossi *et al.*, 2004). This type of evaluation can respond to the issues such as: does the level of knowledge of the workers increase after training; or are the practices of the handlers more adequate after training? In the evaluation of the impact it is essential to prepare a research design that is capable of identifying the results desired by the training that were obtained only due to the program under analysis. In general, in this analysis one or more indicators of results from different groups of individuals are compared, for instance, the level of knowledge of a group before and after training. The design of the research to evaluate the impact can be classified as experimental and quasi-experimental (Rossi *et al.*, 2004).

Program process evaluation assesses whether the program operates as intended (Rossi *et al.*, 2004). In general, the program's organizational plan and the program service utilization plan are assessed. Examples of questions that a process evaluation answers include the following. Are the training program functions adequate? Are the available resources sufficient to support program function? What proportion of the target population is receiving the training? Is the target population satisfied with the training? Notice that program process monitoring is a process evaluation repeated over time.

The process can be analyzed by using different criteria such as administrative, legal, or ethical standards. An evaluation can also compare the operationalization of the program in different locations, or analyze if the program is operating as foreseen in its design (Rossi *et al.*, 2004).

It is recommended that both an evaluation and monitoring of the process as well as an evaluation of the impact of training programs be conducted. It is also noted that other types of evaluation of programs can be pertinent such as the identification of efficiency; this is the relationship between the costs of the program and its results.

4.4   Planning the training programs

The effectiveness of a training program on food safety can be reduced by a lack of understanding of factors that contribute to attaining good results (Egan *et al.*, 2007). In this way, for training in food services advanced planning is needed to achieve success (WHO, 2000). In this planning, it is important to consider the following topics: the target population, issues to be discussed, methods used, time of training, and language used.

4.4.1   Knowing the target public

As part of the planning and implementation of the training programs, the materials and the messages to be presented must be developed in keeping with the target public (WHO, 2000; Jacob *et al.*, 2010). Thus, Jacob *et al.* (2010) recommend: (a) determining the knowledge and the attitudes of the individuals; (b) considering the socio-cultural factors; (c) recognizing the individual perceptions; and (d) identifying suitable means for presenting information. We consider each of these in turn.

(a)   *Determining the knowledge and the attitudes of the target public*: Knowledge and attitudes differ among individuals. For example, some food handlers may have knowledge about issues related to correct handling practices although they do not have practical skills;

these professionals therefore require theoretical and practical training. Others may correctly execute the handling practices, but lack an understanding of why some practices are incorrect; good theoretical education is necessary in this case. In addition, food handlers may have a negative attitude towards correct handling practices, which can require incentives and motivational support to attract improvements in attitude (Seaman, 2010).

(b)   *Consider the socio-cultural factors*: It is important to consider the cultural and social factors of the target public, given that these factors can influence the understanding of the message about food safety (FAO, 1999; WHO, 2000).

(c)   *Recognize the individual perceptions of the participants*: It is important to consider that each individual responds to the information presented in the training in a different way, based on messages that they are able to understand and their experiences (Needleman, 1987), such as past experiences related to food handling (Seaman, 2010).

(d)   *Identify suitable means of presenting information to the population*: Considering the target public is important to identify the best ways to present information referring to food safety (WHO, 2002). In addition, it should be considered that the level of confidence that an individual has in the information source affects the acceptance of the message about food safety (Jacob *et al.*, 2010).

4.4.2   Training themes

The themes raised in training about food safety in food services can differ, above all due to the laws in each location (as addressed in Section 4.2), and as a function of the needs of the establishments (WHO, 2000). Based on the information contained in Table 4.1 it is possible to verify the diversity of the themes addressed in food safety training. Among the issues most raised in the training include 'hygiene', mainly personal hygiene, handwashing techniques, as well as food and workplace hygiene. In addition, information about good handling practices, basic microbiology, foodborne

Table 4.1 Issues presented in food safety training programs. Source: Medeiros *et al.*, 2011. Adapted with permission of Elsevier.

Author, year, country	Issued presented
Costello *et al.* (1997), USA	Food safety, personal hygiene, hand washing, good food handling practices, cross-contamination, cleaning procedures/washing, and sanitization
Lillquist *et al.* (2005), USA	Personal hygiene and hand washing
Cenci-Goga *et al.* (2005), Italy	Personal hygiene, food safety, good practices, *Hazard Analysis and Critical Control Points*, cleaning procedures/washing, and sanitization
Capunzo *et al.* (2005), Italy	Personal hygiene, good practices, *Hazard Analysis and Critical Control Points*, basic microbiology, personal behavior, and utensil hygiene
Danchaivijitr *et al.* (2005), Thailand	Gastroenteritis and food handling practices
Finch and Daniel (2005), USA	Foodborne diseases, evaluation of food product safety, and food safety
Salazar *et al.* (2005), USA	*Hazard Analysis and Critical Control Points* and food safety
Quaranta *et al.* (2007), Italy	*Hazard Analysis and Critical Control Points*, basic microbiology, checklist, food quality, nutritional quality, and self-control
Pollitt (2007), UK	Personal hygiene, good practices, work hygiene, personal behavior, menu management, client service, and company policies
El Derea *et al.* (2008), Egypt	Food handling practices, personal hygiene, and hand washing
Malhotra *et al.* (2008), India	Personal and workplace hygiene, basic microbiology, foodborne diseases, and water and food hygiene
Acikel *et al.* (2008), Turkey	Personal hygiene, hand washing, and food hygiene
Winter *et al.* (2009), USA	Songs presented: 'You'd better wash your hands', 'They might kill you/we are the microbes', 'Stayin' alive' and 'Don't be a gambler'
York *et al.* (2009), USA	Food safety, hand washing, use of thermometer, and handling of work surfaces
Park *et al.* (2010), Korea	Basic microbiology, measuring temperature, hand washing, personal hygiene, cleaning the location, good handling practices, and safe purchase and storage
Choudhury *et al.* (2011), India	Personal hygiene, food hygiene, health and nutrition, workplace hygiene, and specific products
Ababio (2011), Ghana	Food hygiene, personal hygiene, and introduction to national and international food laws
Rajagopal (2012), USA	Foodborne diseases, food handling, hand washing techniques, and use of gloves

diseases, and food safety are topics frequently included in training.

Considering that it is important to make a good presentation of all training issues, care should be taken to avoid including too much information in the training program. If necessary, it is recommended that a larger number of training sessions about food safety be conducted so that the population assimilates the information better, as achieved in the trainings conducted by Choudhury *et al.* (2011).

4.4.3 Training methods

The type of method used in the training programs can influence changes in behavior, attitude, acceptance, and satisfaction of the participants (Medeiros *et al.*, 2011). According to a review of the work published from January 2004 to April 2009, Medeiros *et al.* (2011) evaluated the methods used in food safety training for food service workers. For the authors, the methods that had the best acceptance among the food handlers

were audiovisual resources, interactive media, and lectures.

As can be seen in Table 4.2, the joint use of different methods in food handler training is a frequent practice. Nevertheless, there is little research that compares these methods (Medeiros *et al.*, 2011) as does the work of Costello *et al.* (1997) and Lillquist *et al.* (2005). The study conducted by Lillquist *et al.* (2005) to evaluate the impact of training found that the participants in interactive training about hand washing had significantly better results in the knowledge test on the training day and on the retest of knowledge (two weeks after training) when compared to the group that only had access to lectures and videos and the control group (The study divided the employees into three groups: Group I did not receive training; Group II was trained with a lecture and videos and responded to a written test. Group III was trained with a lecture, videos, practice with hand washing, and responded to a written test. The question referring to the best strategy to obtain more information from the training was presented only for the participants of Groups II and III). Costello *et al.* (1997) compared training via lecture method with a computer-assisted interactive method and found that both methods led to an improvement in the knowledge of food handlers.

Based on this, it was noted that different training methods can be used to teach people about food safety. These include: lectures; use of audiovisual resources and interactive media; use of printed material; preparation and execution of recreational and practical activities; among others (Table 4.2).

4.4.3.1   Lecture

The scientific literature indicates that the use of the lecture method is very frequent in food safety training programs (e.g. Costello *et al.*, 1997; Capunzo *et al.*, 2005; Danchaivijitr *et al.*, 2005; Lillquist *et al.*, 2005; Quaranta *et al.*, 2007; Malhotra *et al.*, 2008; Park *et al.*, 2010; Ababio, 2011) (Table 4.2). This method allows various people to be trained at a single location at the same time. This can result in lower training costs, and allows greater interaction among participants (DiPietro 2006).

Table 4.2   Methods and results of food safety training programs. Source: Medeiros *et al.*, 2011. Adapted with permission of Elsevier.

Author, year, country	Food safety training		
	Participants	**Method**	**Main results**
Costello *et al.* (1997), USA	Employees of quick-service restaurant	Lecture and interactive computer	Participants displayed improved knowledge. Better retention of knowledge was found among those who received training with computers
Singh (2004), India	Food handlers in hospital food service	Audiovisual resources and practical hand-washing techniques	An increase in knowledge about food safety and hand-washing practices was observed
Lillquist *et al.* (2005), USA	Food handlers	Videos, lectures, writing and reading techniques, and hand-washing practices	The individuals who participated in the hand-washing practices had statistically better results on the knowledge test when compared to individuals who did not have this practice
Capunzo *et al.* (2005), Italy	Food handlers on board ships	Lectures, audiovisual resources, slides, illustrations, comic strips, and posters	After training, contamination by total bacteria, *total coli* and *fecal coli* decreased on utensils, in storage areas, furniture, and on refrigerators in the establishment

(Continued)

Table 4.2 (Continued)

Author, year, country	Food safety training		
	Participants	Method	Main results
Danchaivijitr *et al.* (2005), Thailand	Food handlers in hospital food service	Readings and handouts	After training there was a significant decrease in the prevalence of stool pathogens and parasites among the food handlers, although improved knowledge and hand-washing practices were not observed among these professionals
Salazar *et al.* (2005), USA	Food handlers in university food service	Videos, workbook, posters and activities using magnets, thermometers, gloves	There was a significant increase in knowledge among participants after the training
Quaranta *et al.* (2007), Italy	Food handlers at corporate restaurants	Lecture, slides, and DVD	An increase in knowledge among the participants was observed after the training
Pollitt (2007), UK	Employees of fast-food restaurant	Recreational activities, games, music, balloons, and booklets	92% of the participants considered the booklets to be a very informative and useful to learn about the issues presented in the training
El Derea *et al.* (2008), Egypt	Food handlers at hospital food service	Folders, posters, and visual resources	There was an improvement in the bacteriological quality of the meals served after training, and a significant increase in the knowledge of the food handlers in most of the issues presented
Malhotra *et al.* (2008), India	Food handlers at medical college establishments, excluding hospital kitchens	Lecture using flip chart and posters	After training, there was a significant increase in knowledge among participants about hand washing and nail cleaning. There was a positive change in attitude of the handlers about the use of a uniform, and hand washing after using the bathroom
Acikel *et al.* (2008), Turkey	Employees of hospital food service	Practical hand-washing techniques. Material of the Gulhane Military Medical Academy Department of Public Health	After training, there was a significant reduction in the use of watches and jewelry, and a significant increase in knowledge among participants
Winter *et al.* (2009), USA	Supervisors of food service school	Musical parodies	The messages contained in the songs were remembered by 96% of the supervisors in food service schools and by 81% of the managers of food service schools
York *et al.* (2009), USA	Restaurant employees	ServSafe Employee Guide and supporting materials	There was a significant increase in knowledge about hand washing because of training, and greater frequency in the practice of this activity

(Continued)

Table 4.2   (*Continued*)

Author, year, country	Food safety training		
	Participants	**Method**	**Main results**
Park *et al.* (2010), Korea	Food handlers in Korean and Japanese restaurant	Lecture and technical demonstration, booklets, microbe plate kit, thermometer, sanitizing detergents, poster	The training allowed a significant increase in knowledge of participants in relation to personal hygiene, and food handling and hygiene
Choudhury *et al.* (2011), India	Street food vendors	Charts, flip charts, posters, motivational videos, dramatizations, demonstration, marionette shows, and booklets	After training, the level of knowledge of the participants increased significantly and there was an improvement in the adoption of good hygiene practices
Ababio (2011), Ghana	Food service employees	Lectures, hand washing practices	There was an increase in knowledge among participants after the training
Rajagopal (2012), USA	Restaurant employees	Posters, videos, power point, hand washing practice, animation to demonstrate use of gloves	The training allowed better knowledge among participants, mainly in relation to hand cleaning and handling foods

The use of this method in training programs led to increased knowledge of food safety among food handlers (Costello, 1997; Lillquist *et al.*, 2005; Quaranta *et al.*, 2007; Malhotra *et al.*, 2008; Park *et al.*, 2010; Ababio, 2011), as well as a decrease in the prevalence of the total bacteria count and total coli and fecal coli on utensils, storage areas, refrigerators, and furniture (Capunzo *et al.*, 2005). It is common to accompany a lecture with other methods (Table 4.2), such as slides (Capunzo *et al.*, 2005; Quaranta *et al.*, 2007), practical activities (Lillquist *et al.*, 2005; Park *et al.*, 2010; Ababio, 2011), and videos (Lillquist *et al.*, 2005; Quaranta *et al.*, 2007).

4.4.3.2   Audiovisual resources and interactive media

Considering the scientific literature, it can be seen that audiovisual resources and interactive media are frequently employed to explain food safety issues in trainings (Table 4.2). It is important to consider that, although the initial cost of the use of

these methods can be high (due to the cost of production or purchase of the materials and equipment), the materials can be used at other times leading to a lower cost over the long term (DiPietro, 2006). Such resources include videos (Lillquist *et al.*, 2005; Salazar *et al.*, 2005; Quaranta *et al.*, 2007; Choudhury *et al.*, 2011), slides (Capunzo *et al.*, 2005; Quaranta *et al.*, 2007; Rajagopal, 2012), music (Pollitt, 2007; Winter *et al.*, 2009), and computer software (Costello *et al.*, 1997).

The use of videos about food safety in food service training has increased considerably in recent years (Table 4.2). This method has also been used by government agencies (FSA, 2012). Analyses of the results of training programs that include videos to present information indicated improved knowledge among the food handlers and better food handling practices in the establishments (Lillquist *et al.*, 2005; Salazar *et al.*, 2005; Quaranta *et al.*, 2007; Choudhury *et al.*, 2011).

The use of slides in training as part of the methods employed has been common, mainly in conjunction with lectures and videos (Table 4.2). Capunzo *et al.* (2005), Quaranta *et al.* (2007) and Rajagopal (2012) had good results with training of food handlers in food service in which slides were displayed to present information. One other resource also included in the planning of the training is the use of music (Table 4.2). Winter *et al.* (2009) included in a training program four parodies of songs with food safety information ('You'd better wash your hands', 'They might kill you/we are the microbes', 'Stayin' alive' and 'Don't be a gambler), and noted that the messages contained in the songs were remembered by a large portion of the participants. A similar strategy was also used by Pollitt (2007) when planning training for fast-food services employees. This author also used music, in conjunction with other resources, to present training messages to employees (Pollitt, 2007). Considering the little scientific evidence about the use of this method in food safety training, it is recommended that music be used as a complementary method (Winter *et al.*, 2009).

The preparation of computer software and its use in training of food handlers can be considered another option. However, the scientific literature indicates that the use of this resource is still not frequent (Table 4.2). In a study conducted by Costello *et al.* (1997), the employees of fast-food establishments that conducted training with computers increased their knowledge about the issues addressed, and retained 100% of the knowledge acquired. In addition to the good results found in the study, training by means of computer software can be conducted individually, thus not harming meal production at the establishment even when it is conducted during work hours (Costello *et al.*, 1997).

4.4.3.3   Printed materials
Published studies indicate that printed materials are among the most frequent methods used in food safety training (Table 4.2). The literature highlights that the provision of printed materials allows participants to have the information even after training (Purcell *et al.*, 2010). The printed materials include booklets (Danchaivijitr *et al.*, 2005; Pollitt, 2007; Choudhury *et al.*, 2011), pamphlets (El Derea *et al.*, 2008), posters (Capunzo *et al.*, 2005, Salazar *et al.*, 2005; El Derea *et al.*, 2008; Malhotra *et al.*, 2008; Choudhury *et al.*, 2011; Rajagopal, 2012), infosheets (Chapman *et al.*, 2011), illustrations and comic strips (Capunzo *et al.*, 2005; Choudhury *et al.*, 2011), and flip charts (Malhotra *et al.*, 2008; Choudhury *et al.*, 2011).

In addition to being well accepted by the participants (Pollitt, 2007; El Derea *et al.*, 2008), the inclusion of printed materials in the training programs also helps to increase knowledge (Salazar *et al.*, 2005; El Derea *et al.*, 2008; Malhotra *et al.*, 2008; Choudhury *et al.*, 2011; Rajagopal, 2012). The printed materials are frequently used in conjunction with other resources, as can be seen in Table 4.2. In addition to using audiovisual resources, slides, illustrations and comic strips in the face-to-face lessons conducted, Capunzo *et al.* (2005) placed didactical posters with images and descriptions of some operational instructions in the most critical areas of the food handlers' work environment. In the training programs for food handlers developed by Malhotra *et al.* (2008), lectures were conducted using a flip chart and posters. Choudhury *et al.* (2011) used different types of printed materials in conjunction with dramatizations, videos, and marionettes.

In a project conducted by Chapman *et al.* (2011), the authors prepared infosheets to be used in food handler training. These materials differ from others used given that this communication tool was based on verbal histories and narratives that describe real foodborne disease outbreaks (Chapman *et al.*, 2011). Some authors believe that this method can be more effective in the transmission of information than descriptive messages or the use of numerical statistics alone, given that the food handlers identify themselves with the verbal stories or narratives, making the information relevant to their lives (Powell *et al.*, 2002).

It is important to consider that a large quantity of materials such as brochures, manuals, pocket

guides, and other documents about food safety are available on official sites. Among the sites that provide information, that of the World Health Organization is recommended (http://www.who. int/en/). The documents have a scientific basis, they are written by experts, and some are published in more than one language (e.g. both English and Spanish), which allows access by a greater number of people. In addition, the use of materials prepared by agencies responsible for health control and inspection from the location where the food safety training will be conducted is recommended.

4.4.3.4   Recreational activities and practices

After analyzing some scientific publications, it was noticed that the preparation and execution of recreational activities and practical actions are also present in food handler training (Table 4.2). Among the activities identified in the scientific literature are: reading activities with the participants (Lillquist *et al.*, 2005; Salazar *et al.*, 2005); games (Pollitt, 2007); practical activities related to hand washing (Singh, 2004; Lillquist *et al.*, 2005; Acikel *et al.*, 2008; Rajagopal, 2012); and the use of gloves and thermometers (Salazar *et al.*, 2005).

Practical activities with hand washing are one of the most common in food services training (Medeiros *et al.*, 2011). This practice is well accepted by the food handlers, yielding positive results in food safety training (Singh, 2004; Lillquist *et al.*, 2005; Acikel *et al.*, 2008; Rajagopal, 2012). According to Lillquist *et al.* (2005), the cost of including this activity is small given that the materials used such as soap, water, and paper towels are relatively inexpensive and/or are already available at the establishments. In addition, the time needed to incorporate this practice is small; in approximately 7 minutes it is possible to conduct a demonstration and have practical participation in hand washing (Lillquist *et al.*, 2005). Practice with the use of gloves and thermometers, although only observed in the study by Salazar *et al.* (2005), also deserves to be mentioned given that the incorrect use of these utensils can affect the provision of safe food (Lynch *et al.*, 2005; FDA, 2009).

4.4.4   Duration of training programs

Some studies discuss the relationship between the length of training programs and the training results. Medeiros *et al.* (2011) observed in their review that the greater the time of employee training (e.g. 5 hours or more) the greater the amount of knowledge obtained. Interventions of 4 hours or less also led to advances in knowledge and attitudes by food handlers, although not all the improvements were significantly different. It is therefore recommended that the training be at least 4 hours, which can be divided into a number of sessions (York *et al.*, 2009; Choudhury *et al.*, 2011). In addition, it is essential to consider the recommendations of the norms and laws in each city, state, and country.

4.4.5   Language used in training

Seeking to achieve a better understanding of the information presented, some authors provide recommendations about the style of language to be used in food safety training (e.g. Quaranta *et al.*, 2007; Jacob *et al.*, 2010). It is important that the information presented in food safety training be clear, coherent, direct, and suitable for the target public (Quaranta *et al.*, 2007; Jacob *et al.*, 2010). If it is not, the information may be ignored by the participants (Jacob *et al.*, 2010). In addition, the messages presented in the training programs must be consistent, that is, when using different strategies to present information it is essential that there are no conflicting messages (Powell *et al.*, 2002).

4.5   Conclusions and future outlook

Careful planning is important in the development of an effective training program for food handlers in food services. It is first important to investigate the regulations at each location, which can determine the need for specificities in the training program. It is also recommended

that the characteristics of the target population at which the program is aimed be considered. It is important to guarantee the use of clear and coherent language during training, in order to help participants understand. Training programs often use more than one method. Audiovisual resources, interactive media, printed materials, and recreational activities and practices are used in conjunction with the lecture. Hand-washing practices stand out among the practical activities, due to the low cost and because they are well accepted by the training participants, resulting in positive training. It is important that an evaluation of a training program be made to determine if the objectives were achieved and if the program is operating suitably. It is believed that although the focus here was on studies about food handlers in food services, the recommendations presented can serve as a basis for training programs for food safety with other types of target public such as consumers.

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5
Product Tracing Systems

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Summary

The ability to rapidly and effectively trace food products is critical during a food-related emergency, such as needing to determine the cause of an outbreak or needing to remove food from the supply chain during a recall. Regulatory traceability requirements vary widely, and the perception that traceability requires investment without resulting benefit means that the capabilities of supply chain members to trace are inconsistent. Global requirements, including governmental and industry drivers are discussed. The various ways that technology can be utilized, and the issues around costs and benefits, are presented in the context of identifying how a global product tracing system can be realized.

5.1 Introduction

In Western societies, and increasingly in developing economies, grocery stores carry food from around the world. Many processed and other multi-ingredient foods consist of components sourced from all corners of the globe. History shows that food safety issues can occur at any point along the supply chain and, the earlier in the supply chain that uncontrolled hazards are introduced, the greater the potential spread of an issue. However, when a contaminated ingredient results in illnesses associated with different products for example, what are the systems in place for tracing ingredients back through the supply chain to determine the common cause? Currently the product tracing systems that exist on a global scale are inadequate to follow the path of a food from origin to consumption and back, and even when the documentation exists that enables this trace, rarely if ever can it be done in a timeframe sufficient to protect public health.

The inadequacy of product tracing systems stem from a number of issues, each of which will be addressed in this chapter: a lack of understanding

of what it means to trace a product; weak international standards around product tracing; lack of harmonized expectations for traceability between multiple geographies; incomplete articulation of industry- and company-specific benefits of improved recordkeeping; non-standardized technological 'solutions'; inability to define the goal; and competing priorities.

5.2   Traceability: meaning and context

The term 'traceability' means different things to different people in different contexts. In the European Union, traceability is linked to genetic origin, and is often referenced in discussions around genetically modified organisms (GMOs) (Miraglia *et al.*, 2004). In the international context, traceability also refers to the ability to distinguish products at a molecular level (Picarro Inc.). Laboratory standards also use the term 'traceability' to mean an unbroken chain of measurements, each with defined uncertainty, that can be traced back to an official standard measurement (National Institute of Standards and Technology, 2012).

For these reasons, the term 'product tracing' has been used preferentially, or at least synonymously, with traceability when discussing the ability to trace a food product, and its constituents, forward or backward through the supply chain. As related to determining the path of a food product, some commonly accepted definitions include the following.

Traceability is defined in the eLogistics glossary as: '…the registering and tracking of parts, processes, and material used in production, by lot or serial number' (http://www.elogistics-trendwatch.com). An often quoted definition comes from the now-withdrawn 1994 International Standard ISO 8402:1994 Quality management and quality assurance, where traceability is defined as: 'The ability to trace the history, application or location of an entity by means of recorded identifications' (International Standards Organization, 1994). The more recent 2000 International Standard ISO 9000:2000 Quality management systems has a less

specific definition of traceability: 'The ability to trace the history, application or location of that which is under consideration' (International Standards Organization, 1995).

When Trautman *et al.* (2008) reviewed the literature around food product tracing, they found over 30 definitions of the term 'traceability'. In general, traceability is understood as the ability to follow the movement of a food product through the stages of production, processing, and distribution, both backward and forward. 'Traceback' is the ability to trace a food product from the retail shelf back to the farm. Conversely, 'traceforward' is the ability to trace a food product from the farm forward to the retail shelf (Levinson, 2009). More recently, traceability has been distinguished from product tracing, with traceability often being recognized as the practices within a single firm; product tracing is the supply-chain-wide system that provides for trace back and forward. However, these are not universally accepted definitions but instead reflect the sentiments of industry members who state 'my company has great traceability'; conventional definitions suggest that a single company cannot in and of itself have traceability, since this traceability spans supply chain members. Clarity around terms is therefore needed.

5.2.1   Tracebacks, traceforwards, and recalls

Although traceability is predominantly an exercise in recordkeeping, its importance is most notable during food safety events. In an outbreak scenario, epidemiologists seek to focus on the potential foods that might be causing illness. When cases occur in disparate geographies, it is the traceback effort that can determine if foods consumed at different locations stemmed from a common origin, whether a common point of storage, manufacture, harvest, or even a common ingredient. The traceback process is therefore said to seek a 'point of convergence' where all the distribution paths cross. As described in the following, these paths are seldom linear and are often very bumpy. There are inconsistencies in the type of information captured

at each point in the supply chain, the way it is captured, and the ease with which is it accessed and linked to the next point in the supply chain. Performing tracebacks through the manual collection and assimilation of records is a time-consuming and laborious effort. Technology affords the ability to more easily analyze records, but the appropriate records must exist in the industry in the first place.

Traceforward investigations are more familiar to most of the food industry than tracebacks. In a traceforward, the origin or source of the food is known. The goal is to determine all possible paths and outlets for the product. Traceforward is synonymous with recall and is typically used to identify, isolate, and remove products in distribution. This removal can be prompted by safety or quality issues.

5.2.2   Traceability system attributes

ISO 22005, which is discussed in more detail in Section 5.4.1, defines a traceability system as the 'totality of data and operations that is capable of maintaining desired information about a product and its components through all or part of its production and utilization chain' (International Standards Organization, 2007). What kind of system can maintain the totality of data throughout the utilization chain? Traceability systems can be established in several ways, where the entity(ies) responsible for providing, accumulating, analyzing, and reporting on the data can differ depending on the construct of the system. In any case, the ISO definition recognizes that traceability is an attribute of a system, not an individual company. The need to trace food products is global, as is the supply chain for many food products. The objectives of a traceability system need to be established, and is most appropriately established by regulators. Golan *et al.* (2003) have suggested that traceability systems can be described based on their breadth, depth, and precision. The accuracy of the system should also be considered. Golan *et al.* state:

> 'Breadth describes the amount of information the traceability system records. Depth is an indication

of how far back or forward the system tracks. In many cases, the depth of a system is largely determined by its breadth: once the firm or regulator has decided which attributes are worth tracking, the depth of the system is essentially determined. Precision reflects the degree of assurance with which the tracing system can pinpoint a particular food product's movement or characteristics. Precision is determined by the unit of analysis used in the system and the acceptable error rate.'

Breadth (how much information the system records) is determined based on the objectives of the traceability system. Key components of such objectives are time, accuracy, and granularity. Regulators or other appropriate bodies must specify the maximum permitted time between when a trace begins and when the origin(s) are identified (based on accuracy, breadth, and granularity). It is reasonable to expect that some situations require a more rapid trace than others. Company- or facility-specific tracing systems, as well as the tracing infrastructure that exists between companies, must be built to respond to the 'worst case' scenarios. The balance between time and accuracy is often seen in company recall plans, which might specify '90% product identified in 4 hours; 100% identified in 24 hours'. As regulators consider appropriate response times, they should also consider that some records are likely more accessible than others, and can be obtained and analyzed more quickly. Finally, there are often concerns about the inability to trace to a very granular level. For example, using modern production techniques it is seldom possible to trace every farm that contributed wheat to a loaf of bread.

5.3   International traceability regulations

Attempting to implement any types of regulations at the international scale is difficult at best. Compared with the other types of food safety issues faced by industries and governments on a regular basis, traceability may not be a priority.

However, on a regular basis, deficiencies in tracing systems demonstrate the need for international cooperation so that the risk to public health does not unnecessarily increase due to a lengthy investigation.

As noted earlier, traceability is an attribute of a system not a company, and the food system is global. The weakest link, anywhere in the world, can therefore compromise the ability to trace a food product. An evaluation of food safety systems around the world shows that the maturity of both the industry and government can vary widely. To date, international product tracing requirements have been very non-descript, and the limited value they provide is further diminished by the inability to enforce the requirements.

5.3.1   Codex

Codex Alimenatarius is the international standards setting body for food issues. Codex members are primarily governments from around the globe, although non-governmental organizations may also participate. Through Codex, governments deliberate and decide upon standards and, in the event of a trade dispute, the World Trade Organization will issue decisions based on Codex Standards.

In some instances, Codex can represent the 'lowest common denominator' because the standards must be constructed in a way that diverse governments agree to, and must be practical and achievable around the world.

Codex has addressed traceability. The lack of agreement between countries immediately becomes apparent when it is observed that Codex uses the terms 'traceability/product tracing'. Codex defines this concept in the Codex Alimentarius Commission Procedural Manual as 'the ability to follow the movement of a food through specified stages(s) of production, processing and distribution' (Codex Alimentarius Commission, 2008). A more detailed explanation of the role of product tracing is found in the Codex document *Principles for Traceability/Product Tracing as a Tool within a Food Inspection and Certification System* (Codex Alimenatarius Commission, 2006). The document identifies that product tracing/traceability can: contribute to the protection of consumers against foodborne hazards; reduce deceptive marketing practices; and facilitate trade on the basis of accurate product descriptions.

This document notes that product tracing does not proactively improve food safety outcomes but must be combined with appropriate measures and requirements. However, it recognizes that product tracing can contribute to the effectiveness and/or efficiency of associated food safety measures. The ability to limit the scope of recalls and respond to food safety incidents more quickly is often seen as a regulatory driver for improved product tracing. The document states that a product tracing tool should be able to identify a product at any specified stage of the food chain (from production to distribution). This can be read in a number of ways, and if interpreted as suggesting that the location of all products is known at all points in time, this could be difficult to achieve. At a minimum, the level of granularity required must be considered (e.g. does this mean that the quantity of each lot code at each location must be known?) as well as the identification of who needs to know (e.g. all supply chain members, all regulators?). This statement can also mean that each supply chain member maintains control of the product and has visibility into the products that are under their control and/or ownership.

The Codex document is consistent with the current approach to traceability in the world (at least in countries where an approach has been articulated). Codex states that food should be traceable with respect to maintaining records of where the food came from (one step back) and where the food went (one step forward) as appropriate to the objectives of the food inspection and certification system. The latter idea of 'as appropriate' provides governments with flexibility in implementation and specification of traceability requirements, but also can contribute to the application of different requirements around the world.

The document notes further that application of traceability/product tracing should take into account the capabilities of developing countries. As discussed in Section 5.5.4, developing countries

are in an interesting position because both their technical capabilities and regulatory capacity vary widely. If an importing country has traceability objectives or outcomes that cannot be met by an exporting country, the importing country should consider how it might assist or support the exporting country, especially in the case of a developing country.

Traceability requirements can be viewed as trade barriers. Food clearly traverses the world, and thus Codex recognizes the importance of establishing a standard for traceability. However, in the short term we are likely to see the disparities in traceability systems and expectations persist.

5.4   Private global traceability standards

Although Codex is often referred to as a 'standards-setting body', any discussion of standards for traceability must specify the objective or purpose of the standard before discussing the standard itself. Much confusion results from discussing 'standards for traceability' as a generic term. Broadly, standards around traceability can be divided into the following categories: standards for identification (of locations, objects, etc.); standards for data sharing (e.g. the format for the transmission of electronic data packets); standards for data encoding (bar codes, RFID tags, etc.); and general expectations.

The Codex 'standard' for traceability sets a general expectation. The implementation of traceability can be facilitated by the other three standards, and these standards are generally associated with private rather than government standards. The only way to improve product tracing in countries lacking strong food safety regulatory bodies is through the pressure exerted by private industry.

5.4.1   International Standards Organization (ISO)

Although ISO is not by definition used to define regulatory standards, some countries, including several in the European Union, do align with or in some cases even adopt ISO standards as part of their regulatory requirements. Still, ISO should be considered a 'private' standard (albeit one that has great influence). ISO 22005 relates specifically to traceability (International Standards Organization, 2007) and, in its introduction, states 'The implementation by an organization of a traceability system depends on: technical limits inherent to the organization and products (i.e. nature of the raw materials, size of the lots, collection and transport procedures, processing and packaging methods); and the cost-benefits of applying such a system.'

The ISO standard for traceability lays out objectives, but provides little if any guidance in how to attain these objectives; it can therefore also be considered a 'general expectation'. The introduction cited above acknowledges the variety of factors that impact the ability of a single facility to trace food products; linking the internal systems that may handle and record data can be challenging, and further relating the traceability information from one company to another presents another layer of challenges.

5.4.2   Global Food Safety Initiative (GFSI)

GFSI is recognized primarily as a benchmarking organization, 'developing a model that determines equivalency between existing food safety schemes, whilst leaving flexibility and choice in the marketplace' (Global Food Safety Initiative). Different audit schemes are benchmarked against a GFSI guidance document which includes, in part, traceability (Global Food Safety Initiative, 2012). The document specifies different requirements for different members of the supply chain but, compared to ISO, is more specific in the expectations of companies. Still, the one-forward, one-back system of tracing is the general expectation within GFSI. The guidance document includes objectives but also, in some instances, specifies the types of information required and is generally more detailed than the Codex or ISO documents. For example, it specifies that food manufacturers must track recycled (e.g. reworked) material, and also states that products must be identified with a unique

code. Although the way this identification is constructed, shared, and captured is still up to the manufacturer, there is increased clarity around the expectation.

Different audit schemes may be more exacting in their traceability expectations, for example by stating timeframes within which certain information must be traced, and/or the frequency with which the tracing system must be tested. Some schemes also emphasize the need to trace specific elements of a food system, such as rework, allergens, and/or packaging.

5.4.3   GS1

GS1 is often mentioned during discussions about traceability. There tends to be substantial confusion among some audiences around the nature, objectives, and approaches of GS1. Part of this confusion may stem from the fact that GS1 is involved in the development of *several types* of standards for traceability, from identification, to data sharing, to standards for bar codes and RFID tags.

GS1 is a global, not-for-profit organization, whose mission is to design and implement 'global standards and solutions to improve the efficiency and visibility of supply and demand chains globally across sectors' (http://www.gs1.org/about/overview). Headquartered in Belgium, GS1 operates worldwide and in-country services are provided by local or regional affiliates called 'member organizations'. Many people mistakenly believe that GS1 is a technology service provider; this is not the case, however.

At the global level, GS1 has developed a Global Traceability Standard which is intended to apply to all kinds of products, not just food (GS1, 2010). This standard expects that users apply the GS1 system for identification, such Global Trade Item Numbers (GTINs) and Global Location Numbers (GLNs), to their traceability systems. It requires users to be able to answer questions in order to be able to trace products, including: who (party identification and data elements); where (location identification and data elements); when (date/time); what (traceable item identification and data elements); and what happened (process or event identification and data elements).

The document provides additional detail and, while it does not specify how the data should be carried or transmitted to supply chain partners, it does provide guidance on the factors that should be considered and proposes an approach that can be taken when developing a traceability system.

GS1 standards for identification are being proposed for use in several industry-driven traceability initiatives, such as the produce traceability initiative and an initiative for the meat and poultry industry (Produce Traceability Initiative, 2013; mpXML). These groups are also advocating for the use of GS1 standards for data carriage (generally the GS1 128 bar code). The stated benefit to this system is that the bar codes can be scanned by each supply chain member, creating the chain that needs to be constructed more manually today. A report by Miller *et al.* (2013) demonstrated how the use of GTIN numbers aided in an outbreak investigation associated with fresh produce, a product type that often lacks such identification.

5.5   Country-specific traceability requirements

With the relative lack of prescriptiveness of both Codex and ISO standards for traceability, it is no surprise that international harmonization around traceability is presently very basic. However, there are two drivers that have motivated some countries to increase their efforts around traceability: food safety and economics (including response to consumer pressures and preferences).

Foodborne-illness investigations present challenges to governments around the globe. While outbreaks rarely make international news, when they do, questions about the efficiency of a government's response abound. A notable example is the 2011 *E. coli* O144 outbreak in Europe which sickened 4075 and included 50 fatalities (World Health Organization, 2011). Determining the source of the outbreak took several weeks and was eventually determined to be Egyptian fenugreek

seeds. Epidemiology and regulatory tracebacks go hand-in-hand and, although this outbreak exemplified deficiencies in the epidemiological investigation, it also highlighted areas for opportunity in traceability.

Another classic example is the 2008 outbreak of Salmonellosis in the United States. Initially, this outbreak was suspected to be associated with tomatoes; examining records associated with tomatoes did not reveal a common point of origin, however. Later, it was found that peppers were the likely cause.

During a crisis governments need to have tools to protect the health of their citizens. However, these outbreaks are rare. A more constant driver to improved traceability lies in economic advantages. In a global economy, companies are not the only ones seeking to build brands. Major exporting countries also want to secure, or grow, their export markets, and traceability can offer a competitive advantage. A strong traceability system can allow countries to more confidently assert the authenticity, and subsequently the safety and quality, of products.

5.5.1   Traceability in developed economies

The regulatory, industry, and consumer views around traceability varies widely and depends on the regulatory structure established in that country and the outbreaks that have caused consumer concern in those countries. Charlebois and MacKay (2010) evaluated food safety systems in 17 peer countries, and performed an evaluation of traceability systems specifically. The assessment focused on regulatory requirements, which may not be reflective of the state of traceability in that industry. In general, countries in the EU and Australia were determined to have favorable traceability systems compared to the US and Canada, which were at the bottom of the ranking for traceability management.

5.5.1.1   United States (US)

Only since 2005 has the United States had any federal traceability requirements. At that time, the 'one-forward, one-back' system of recordkeeping

was required for products regulated by the US Food and Drug Administration (FDA). This essentially requires facilities that manufacture, process, pack, or hold food to maintain records indicating where the product came from and where the product went, and to be able to produce these records within 24 hours of an official request. This approach is aligned with the Codex and ISO standards and, in theory, creates a chain whereby the product can be traced through the supply chain (albeit one step at a time). However, for numerous reasons explored by McEntire *et al.* (2010) and Bhatt *et al.* (2013), this system is not currently effective as evidenced by the tomato/pepper issue of 2008 referenced earlier. Additionally, when records are inadequate or are not produced in a timely fashion, there is generally no penalty for the lack of compliance with this aspect of the law.

With the passage of the Food Safety Modernization Act in 2011, the US Congress authorized the FDA to require additional records to improve the traceability of 'high risk foods'. However, as of the time of writing, this list has not been released by the FDA; based on the issues the FDA must consider in assembling this list, food safety experts can however venture a reasonable guess as to the list of likely candidates.

Not only has the FDA not revealed this list, the FDA has also to specify what the 'additional recordkeeping requirements' will consist of. The FDA conducted pilot studies, as required by the FSMA, to explore methods and technologies that enable more rapid and effective tracking and tracing of products. The report of these pilots contains recommendations, many of which are aimed at improving the FDA's collection of data and ability to analyze data (McEntire *et al.*, 2012). The report also recommends the key data elements (KDEs) that the FDA should require at each critical tracking event (CTE). Table 5.1 contains these recommendations, which includes many pieces of data currently required by many governments around the world and also includes additional data elements that help establish the link between trading partners.

It is important to point out that, at this time, the FDA is not requiring industry to maintain

Table 5.1 Key data elements (KDE) for capture and recordkeeping at critical tracking events (CTE) suggested to the US FDA. R: required field; C: conditional field (the need for this field would be determined by business circumstances; *required in the instance of transport events that do not capture batch/lot numbers); BP: best practice is to capture the batch/lot number or relevant date whenever possible (if difficulty in capturing this information for transport and depletion events, activity ID or other KDEs that provide links, as identified in the table, must be provided* as the industry prepares to meet a future requirement to capture lot/batch numbers). Source: CTE/KDE, FDA task order. Reproduced with permission of Institute of Food Technologists.

Critical tracking events	Transportation (exchange of goods)		Transformation (creation/ manipulation of products)		Depletion (exit from system)	
	Shipping	Receiving	Input	Output	Consumption	Disposal
Currently required KDEs						
Event owner (firm submitting information)	R	R	R	R	R	R
Date/time	R	R	R	R	R	R
Event location	R	R	R	R	R	R
Trading partner [1]	R	R	R			
Item (the good)	R	R	R	R	R	R
Lot/batch/serial#	BP*	BP*	R	R	BP	BP
Quantity	R	R	R	R	R	R
Unit of measure	R	R	R	R	R	R
Linking KDEs						
Activity type (e.g. PO, BOL, work order)	C*	C*	R	R		
Activity ID (number associated with PO, BOL, work order)	C*	C*	R	R		
Transfer type [2]	C	C				
Transfer number [2]	C	C				
Lot/batch relevant date [3]	C	C	C	C	BP	BP
Carrier ID	C	C				
Trailer number	C	C				

[1] In the event of a shipping CTE, the trading partner is the immediate subsequent recipient of the shipment; in the event of a receiving CTE, the trading partner is the immediate previous supplier of the product; in the event of a transformation CTE, the trading partner is the supplier of the input into the transformation.

[2] If the activity type and ID are not linked to a particular shipment of a product (e.g. a purchase order that is fulfilled by multiple shipments over time), then the transfer type and ID are used to indicate the particular shipments that are linked to the activity type and ID.

[3] If there is a different lot/batch designation on a consumer-level product, such as a 'best by' date, it must link to the manufacturer-assigned lot number.

records in an electronic format. Further, the FDA is restricted from prescribing the use of a particular type of technology by the entire food industry. There have been many misconceptions regarding the traceability requirements for food facilities in or exporting to the United States, with most misconceptions suggesting that the requirements are more stringent than they actually are.

The US has attempted to improve traceability in the livestock industry, but the original animal identification program was met with resistance from the industry. A more recent version of the voluntary program provides greater flexibility in the types of identifiers that can be used on animals. The extent to which the program will be adopted remains to be seen.

5.5.1.2 European Union (EU)

The Health and Consumer Protection Directorate-General of the European Commission defines traceability as 'the ability to track any food, feed, food-producing animal or substance that will be used for consumption, through all stages of production, processing and distribution' (European Communities, 2007). It recognizes the need for standardized traceability requirements across all EU member states due to the trade and flow of food products across borders. Within the EU legal framework, certain higher-valued products such as olive oil and honey also have specific traceability requirements to identify their origin and authenticity. Similarly, specific requirements are in place to track and trace foods containing or derived from GMOs. Along with neighboring countries, the EU has also established a rapid alert system for food and feed (RASFF) (European Commission, 2012). This allows regulators to more efficiently share traceability-related data in the event of an outbreak or public health crisis. A similar system called Trade Control and Expert System (TRACES) has been set up to trace animal movements from third countries and within the EU (European Commission, 2013).

Chapter 2/18 of the General Food Law outlines the traceability requirements set forth within the regulatory framework of the EU (European Parliament Council, 2002) as follows.

1. 'The traceability of food, feed, food-producing animals, and any other substance intended to be, or expected to be, incorporated into a food or feed shall be established at all stages of production, processing and distribution.
2. 'Food and feed business operators shall be able to identify any person from whom they have been supplied with a food, a feed, a food-producing animal, or any substance intended to be, or expected to be, incorporated into a food or feed. To this end, such operators shall have in place systems and procedures which allow for this information to be made available to the competent authorities on demand.
3. 'Food and feed business operators shall have in place systems and procedures to identify the other businesses to which their products have been supplied. This information shall be made available to the competent authorities on demand.
4. 'Food or feed which is placed on the market or is likely to be placed on the market in the Community shall be adequately labeled or identified to facilitate its traceability, through relevant documentation or information in accordance with the relevant requirements of more specific provisions.
5. 'Provisions for the purpose of applying the requirements of this Article in respect of specific sectors may be adopted in accordance with the procedure laid down in Article 58(2).'

5.5.1.3 Australia/New Zealand

It is a general requirement in Australia under the Food Standards Code that food businesses must be able to identify where their products (either locally produced or imported) come from. Food businesses are responsible for making the necessary information readily available to the competent authority upon request (Australian Government, 2008). However, such information is not required to be publicly accessible, and there is no prescribed method or format for keeping the records (Yeung, 2011). Food safety practices and general requirements in chapter 3 of the Code covers the 'one step back and one step forward' elements of traceability under Clause 5 (2) Food receipt and Clause 12 Food recall (Food Standards Australia New Zealand, 2012).

There are also special traceability records required for certain food commodities or sectors. For example, a seafood business needs to track and trace the following KDEs (Yeung, 2011): (a) name and address of suppliers or customers; (b) description of the food product; (c) volume or quantity of the seafood received and supplied; (d) batch or lot numbers; and (e) transaction or delivery dates. Some of these requirements stem from minimizing illegal, unreported, or unregulated fishing for environmental and sustainability reasons (in addition to food safety and quality motives).

5.5.2 Traceability through regulatory consolidation

Certain countries are moving towards a more consolidated regulatory framework for food safety and, consequently, for food traceability. They are attempting to unify multiple agencies responsible for regulating disparate food sectors under a single authority.

5.5.2.1 Canada

The Safe Foods for Canadians Act 2012 consolidated the authorities of multiple food commodities to one single agency: the Canada Food Inspection Agency (CFIA). As a part of the Act, CFIA is authorized to adopt additional recordkeeping requirements for improving the traceability of foods. What additional recordkeeping requirements will be enacted is unknown at the time of this writing. Many of Canada's current traceability requirements stem from 'transformative events' as described in Section 5.5.3.1.

5.5.2.2 Egypt

In 2008, Egypt passed new food safety regulations consolidating regulatory efforts under one Food Safety Agency (American Chamber of Commerce in Egypt, 2008). While that law did not consist of mandatory traceability requirements, it did address the conceptual development of product tracing system characteristics and attributes. The expectation was that new requirements would be forthcoming post-creation of this single food safety regulatory authority (American Chamber of Commerce in Egypt, 2008).

5.5.2.3 India

The Food Safety and Standards Act of 2006 is focused on protection of public health (Indian Ministry of Law and Justice, 2006). It attempts to align all stakeholders to prioritize on consumer safety. It also tries to consolidate multiple authorities into a single authority responsible for food safety and adulteration (Sharma, 2013). Under this act, a familiar set of requirements for traceability are put forth. It requires the recordkeeping of one step forward and one step back of all entities that handle foods, similar to the regulations set forth in the US stemming from the Bioterrorism Act of 2002. It also enhances the recall and emergency response system used by regulators in the event of a foodborne outbreak.

5.5.3 Traceability through transformative events

Some countries have enacted or strengthened food traceability regulatory requirements after experiencing transformative incidences or events (from a public health or economic perspective). Canada's BSE outbreak, Japan's tainted rice scandal, and the September 11th terrorist attacks in US are examples of such events.

5.5.3.1 Canada

A Conference Board of Canada report provides a comprehensive review of regulations, attitudes, and opportunities around traceability in Canada (Howard *et al.* 2012). The Canadian government has identified three components necessary for traceability: premise identification; unique product/animal identification; and the recording of movement. To date, traceability in Canada has focused on the livestock industry. Canada has experienced the economic ramifications of BSE, and it is no surprise that the government seeks to track animal movement for the purposes of disease control.

5.5.3.2 Japan

Japan enacted a rice traceability bill in 2010 which consisted of specific country of origin labeling requirements for all food commodities that used rice as an ingredient (Hayashi and Fukuda, 2011). The intent of the law was to foster growth, confidence, and trade in rice produced by Japan. In addition to rice and rice products, Japan also has traceability requirements for domestic beef and requires a degree of traceability for organic foods and genetically modified products. Regarding beef, Japan introduced almost the same traceability system as France and the EU by establishing the Beef Traceability Law in 2003 (Law for Special Measures Concerning the Management and Relay

of Information for Individual Identification of Cattle). Farmers transmit necessary information on their cattle to the MAFF which establishes the individual identification system. All the operators in the distribution chain of beef from slaughterhouses to retailers and restaurants shall indicate the individual identification number of the cattle on their beef to be sold (Takahashi, 2009).

For other foods, Article 3 of Japan's Food Sanitation Law requests that each operator keep records to identify all their suppliers and customers: a 'one-step-back' and 'one-step-forward' record. This request is similar to Article 18 of the European Union's EC Regulation 178/2002. In Japan however, this type of record keeping is only recommended and is not compulsory.

5.5.3.3   South Korea

As a result of a BSE outbreak, South Korea introduced a comprehensive beef traceability system (IUFoST, 2012). 'In general, producers, distributors or retailers of agricultural products may adopt the program on a voluntary basis, and any person who intends to implement the traceability program must first register with NAQS [National Agricultural Product Quality Management Service] … Nevertheless, traceability is mandatory for agricultural products with GAP (good agricultural practices) certification (Yeung, 2011).'

Yeung (2011) further expands on how the South Korean traceability system is implemented.

> 'The traceability information is inputted by the food producers, distributors and retailers into a web-based management information system developed and maintained by the Rural Development Administration, which is a central government agency responsible for agricultural research and services in South Korea. This system is designed to enable the record keeping process and provide consumers with easy and free access to the traceability information. Local authorities responsible for agricultural matters are responsible for promoting the system and providing training to farmers, distributors and retailers. As at 2007, information about 1,400 agricultural products was available through this web-based system. Consumers can use a product's identity

number to retrieve the traceability information via computers, retail store kiosks and [since 2009] their mobile phones. Under this system, consumers can use the 12-digit identity number on the retail beef packaging to obtain information such as the origin, breed and grade of the product through the Internet. Retail outlets can install scanners or other devices to provide traceability information on a voluntary basis.'

5.5.4   Traceability in developing countries

In what seems like a paradox, some of the more aggressive traceability initiatives are occurring in developing economies. There are several reasons for this. Exports are often a large part of developing economies, and countries want to stake their claim to a portion of the export market by demonstrating superior traceability. As the physical infrastructure is developed, there are opportunities to literally lay the foundation for traceability as communication systems are developed and technology is implemented. In countries with maturing regulatory structures, traceability can be written into requirements rather than being included as afterthoughts, and viewed as additional requirements by the regulated industry. Finally, as companies form and grow, traceability can be integrated into systems, processes, and facilities, rather than needing to be added as a retrofit.

5.5.4.1   China

No one can deny China's influence in the global economy. Seafood and canned fruits and vegetables are recognized as major exports, but many underestimate the amount of food ingredients and raw materials that are also produced in China.

Recognizing their role, and the perceptions associated with Chinese food products (based in part on issues with melamine in pet food and infant formula), China stressed the importance of traceability in their most recent 5-year food safety plan (Delegation of the European Union in China, 2011). The plan asserts that China will prioritize traceability efforts based on food type, with wine, dairy, health foods, and other products

for which the Chinese have concerns constituting the first products to be subject to increased traceability requirements.

China has already conceptualized their comprehensive approach to the safety of health food products, and plans to build a national database of traceability information collected from manufacturers. China will pair this information with consumer complaints, product testing data, and other information in order to quickly identify problems and be positioned to rapidly recall a product (Delegation of the European Union in China, 2011).

5.5.4.2   Thailand

Seafood is a major export for Thailand earning over $US1.27 billion from international sales annually (Thai Portal Co., 2011). In 2010,

> 'Thailand's Ministry of Agriculture and Cooperatives (MOAC) partnered with IBM, FXA Group, and the Communications Authority of Thailand (CAT) to implement a global traceability program, allowing the country's exports to be tracked from the retail level all the way back to the farm. The pilot program, which only applies to processed chicken and mangoes destined for export, will use smart sensor technology and traceability software to allow 'all participants in the food chain' to access information on the products, including farm of origin, date of harvest, and temperature during shipping.' (Bottemiller, 2010)

According to the Thai Agricultural Standards Act of 2008, a traceability/product tracing tool should be able to identify at any specified stage of the food chain (from production to distribution) where the food came from (one step back) and where the food went (one step forward), as appropriate to the objectives of the agricultural commodities and food inspection and certification system (Thai Ministry of Agriculture and Cooperatives, 2008).

5.5.4.3   Brazil

Brazil's traceability practices have continued to evolve, particularly in the meat sector where Brazil is a major player. Traceability is required at all export quality abattoirs in Brazil, with system specifications that are consistent with the demands of the European Commission. The Brazilian system provides the following information: name and address of the cattle grower; sanitary control number; country of birth; growing and slaughter information of stock; cut name and date of slaughter; shelf life; traceability code (the same number as the sanitary control number); production date; batch number; gender; and approximate age of the cattle.

5.5.4.4   Jordan

Another country that is pushing for traceability due to trade benefits (in addition to food safety advantages) is Jordan. It has specified traceability requirements for imports. According to Spring Singapore (2010), 'Jordan has a very advanced national digital database for tracking imports based on mandatory registration of food firms importing food into Jordan'. However, there are no domestic traceability requirements.

5.5.4.5   Namibia

Namibia requires the electronic database tracking of all livestock, a mandatory requirement as per the Animal Identification Regulations GN29/2009 (NamLITS Online, 2008). According to its website, some of the benefits identified for such a traceability system include improved: disease surveillance; emergency management; food safety; food quality; and international trade.

5.5.4.6   Singapore

According to the report by Yeung (2011),

> '…although traceability requirements are not specified under Singapore's food acts, the registration and licensing systems in place play a certain role in product tracing… Food manufacturing and processing establishments are encouraged to take a proactive approach to ensure food safety by establishing documentation and record keeping under the Food Factory Grading System. A food establishment's practices in specific food safety areas such as putting in place food recall procedures and maintaining product and process control monitoring records, and food supplier and distribution records are among the assessment criteria for [Agri-Food and Veterinary Authority] AVA's grading inspection.'

5.5.4.7   Taiwan

Again, Yeung (2011) describes the regulatory framework for traceability requirements in Taiwan:

'Under Article 7 of this [Agricultural Production and Certification] Act, [Council of Agriculture of the Executive Yuan] COA implements a voluntary traceability certification system on certain domestic agricultural products, and if necessary, COA may make announcements about the items and scope of specific agricultural products (including imported products) which are required to comply with compulsory traceability standards … Consumers may get access to the relevant traceability information by keying in the product's traceable code on the [Taiwan Agricultural Food Traceability System] TAFTS website, or at kiosks in supermarkets and shopping centers. However, agricultural product operators are allowed to adopt TAFTS on a voluntary basis. Nevertheless, COA has set a goal of implementing mandatory traceability in all agricultural products by 2015, so as to meet the food safety requirements for exporting agricultural products to some foreign markets, such as the European Union and Japan.'

5.6   Costs and benefits to traceability

From the perspective of the food industry, traceability is often viewed as a cost with no identifiable benefit. Attempts at estimating this cost have been largely unsuccessful, given that traceability takes many shapes and sizes (Mejia *et al.* 2010; McEntire *et al.*, 2012). Most cost-benefit studies are very specific, looking at particular firms using particular technologies.

5.6.1   Societal benefits

Public health benefits are the easiest to quantify. In the United States, fewer than half of foodborne illness outbreak investigations are resolved (Center for Science in the Public Interest, 2011). This means that either the pathogen or the food product (or both) was not identified. As government resources dwindle, the number of solved investigations has decreased. There may be many obstacles to a successful investigation, but in instances where the product is not identified traceability could be a factor. The food safety community will not be as effective at putting mitigation in place if the causes of most outbreaks go unknown. Better traceability should help solve more investigations. This also results in greater accountability, but the impact on public health should be measurable.

A report by McEntire *et al.* (2012) calculated the potential health-associated savings if the traceback time had been shortened for a number of well-established outbreaks for which data were available, including the dates that individuals became ill, the dates that tracebacks were initiated, etc. The cost saving was dependent on the outbreak and the food product, with short-shelf-life products generally demonstrating a decreased ability to impact health-associated costs (primarily because the short shelf life of the product means that the traceback may not have started until after the product had already exited the system). Still, the approach showed that shaving days off the amount of time spent on tracebacks could yield public benefit, with the potential savings for some outbreaks reaching more than $US10 million and with rapid tracebacks reducing the number of ill individuals by more than half.

5.6.2   Government benefits

During outbreak investigations, governments use resources (generally tax-payer dollars) and better use of resources is always a benefit. Slow responses and ongoing outbreaks diminish trust in the government (as well as industry). The inability to consistently solve outbreak investigations was mentioned previously; the government would benefit from more completed investigations in the sense that regulations could be enacted that address known issues.

5.6.3   Industry costs and benefits

There are two main categories of benefits that can be assigned to the food industry. The first category is difficult to quantify and includes the ability to protect the value and reputation of a commodity

and a particular brand when an outbreak is associated with a type of product. This relates directly to consumer confidence in the food supply but is difficult to attribute to a particular company and is difficult to measure.

The second category of benefits includes those that are easier for an individual company to quantify, such as efficiency (including reduction in shrink and increase in accuracy of batching and shipping), the ability to market to customers, evaluation of suppliers, and substantiation and verification of claims (such as organic, sustainable, etc.). Companies also look to better traceability to help them manage a recall. Ideal traceability will allow a company to quickly account for all potentially affected products. Currently, some internal traceability systems are designed such that accuracy is compromised, resulting in a determination that 'most of the product went here, but some could have gone there or there, so we need to recall anything produced over the course of these days (or on these lines, etc.)'.

It must be recognized that, due to the tremendous variation in the systems, processes, and capabilities of companies today, some companies may be able to calculate great benefits associated with improved recordkeeping while other companies may have already derived these benefits in other ways that are unrelated to improved traceability. It is therefore impossible to state that any and all firms changing from 'System A' to 'System B' will experience the same costs and same benefits. Additionally, different parts of the supply chain, from growers to manufacturers to distributors to retailers, will bear a different fraction of the cost and will also derive different benefits.

Each company will experience a different set of costs, and the magnitude of the costs will vary. Mejia *et al.* (2010) describe the various categories of costs associated with tracing. In many instances, the costs are related to the types of technologies employed to improve tracing, as described in Section 5.7.2. Some costs may relate to capital equipment and investments in hardware or software; other types of costs may be ongoing, related to the need to continually capture data.

5.7   Challenges

Regardless of whether or not there is regulatory alignment around traceability requirements at an international scale, there are still substantial barriers faced by food industry members around the globe. Commodity-specific challenges are addressed in Section 5.7.3, while the following section highlights some general issues that will need to be overcome in order to enable food traceability.

5.7.1   Education

Technical and economic issues will only be overcome when food manufacturers and others in the supply chain have an appreciation for the role traceability plays as part of a larger food safety management system. Those in the food supply chain need an understanding of how tracebacks differ from recalls, as well as the opportunities for increased supply chain management. This results in an increase in product quality and improved operational and supply chain efficiency. Additionally, awareness of regulatory requirements is a necessity that is often taken for granted. Education in why traceability is important, from the perspective of the CEO to the line worker, is needed if we are to accomplish the goal of a fully traceable food supply. Old habits die hard and the statement 'but we've never had a recall' provides no guarantees for future performance. Firms must be fully aware of the risks their brands are exposed to when inadequate tracing systems are in place.

5.7.2   Technology

Worldwide access to different types of technology, and the communication infrastructure in different parts of the world, varies widely. It is unreasonable to expect a fully electronic traceability system when products are grown in regions of the world that lack electricity. Although the prevalence of cell towers continues to grow there are still regions, including regions of Western countries, that lack this communication capability.

5.7.3 Commingling: a challenge to traceability

Using the phrase 'supply chain traceability' implies a homogeneity to the supply chain that does not exist. While industries seem to like to segment themselves based on the products they produce, from the standpoint of traceability it might be more logical to group challenges by processes rather than by other product characteristics.

Whether grains, oil, sweeteners, dairy, tomatoes, or ground beef, commingling results in decreased granularity of traceability. The issue of commingling is often pointed to by food industry members seeking to demonstrate their inability to trace products. Mixing of any sort, including the mixing of ingredients to form another product, certainly complicates the ability to accurately identify the source of a product. It is not possible to pinpoint the dairy that a drop of milk came from. However, there are ways to decrease exposure when commingling is part of an operation.

Few equate commingling of commodities with the manufacturing of processed foods; the complexities of product tracing are quite similar, however. During the manufacture of food products, companies control the size and definition of a 'lot'. While there are numerous factors that come into play when defining a 'lot' of finished product, one factor is the risk or exposure the firm is willing to face in the event of a recall. If there is a need to remove the product from the marketplace, the removal of a small volume or small number of units is less costly than the removal of a large volume. Similarly, a silo (or other bulk storage) can be thought of as the new lot, comprised of multiple inputs. It is important that companies that handle commingled product carefully consider the frequency with which there is a 'clean break'. For example, in the United States it is required that milk silos are emptied every 72 hours. This means that although numerous tanker trucks of milk, sourced from even more dairy farms, are present in the dairy silo at one time, every three days there is a clean break. An issue with milk cannot therefore be easily associated with a particular dairy farm, but the range of possibilities is controlled to those from which milk was provided during that 72-hour period. Some types of silos are emptied and cleaned much less frequently. It is up to a firm to weigh the benefits of continual loading of a silo versus the benefits of limiting the potential sources of a problem.

5.8 The role of technology in traceability

The role of technology in traceability can be segregated into two overarching categories: how to use technology and why we use technology in traceability. Technology is primarily used to conduct the following four tasks as it is related to traceability.

1. *Data encoding*: In large-scale complex operations, technology is a necessity to batch or lot code generation. The ability to generate globally unique product identities at any granularity (pallet, case, or item) generally requires the adoption of a formal (standardized) coding system. Once KDEs are generated, they need to be encoded onto the product in some way. Most often, this is done through the use of certain types of bar coding systems. Technology is used to generate these bar codes (either through label printing that is later attached to the product or through in-line printing). Technology is also used to define the types of KDEs that would be encoded onto a bar code. With limited space, some KDEs could be stored in an external database and be referenced (or linked) via a primary key that is then encoded into the bar code. Radio frequency identification (RFID) tags are more sophisticated types of data encoders and carriers.

2. *Data capture*: Once KDEs are encoded, they need to be captured and stored at every CTE. There are numerous technologies that enable and streamline the process of capturing data. From bar code scanners to RFID tag readers, the purpose of data capture is two-fold: first,

to create a historical record of the event; and second, to enable linking one event to another. For example, when a batch of ingredients is 'received' at a processing facility, the fact that this event occurred needs to be captured. Subsequently, this receiving event needs to be linked to any transformation (use of ingredients to make finished product) or shipping (sale of ingredient or finished product to customer) event that occurs within the processing facility. There are also several options for storing captured data. The data could be stored locally within a facility where the event is occurring, could be stored externally at the corporate headquarters or data center, or could be stored on the cloud using third-party software-as-a-service providers.

3. *Data sharing*: Once the data have been captured, there are several scenarios when it needs to be shared. From a normal business operations perspective, captured data would need to be shared with employees and management of the facility or corporation. This includes informing company drivers of what's in the truck or informing management of just-in-time inventory loads. Almost always, subsets of data also need to be shared with suppliers and customers. This includes providing a purchase order number to a supplier or an invoice number to a customer. Finally, a subset of data would need to be shared with regulators upon request. The request could be due to a foodborne outbreak investigation or through routine inspections. Along similar lines, traceability data would also need to be shared during mock recalls. Technology enables a faster and more efficient method for transferring and receiving data in electronic format. Strict access controls are put in place to alleviate any concerns about unauthorized access by members of the supply chain, competitors, or regulators.

4. *Data analysis*: Finally, and perhaps most importantly, all the data encoding, capturing, storing, and sharing needs to be analyzed. This analysis could include looking for trends or deviations from the norm. This analysis could be to conduct risk assessments and minimizations across multiple suppliers and customers. Strong data analytical and visualization tools are powerful aids to decision-making from business and crisis management perspectives. Several technologies exist for the sole purpose of mining all the historical traceability and food safety data to predict the behavior, robustness, and weaknesses in a supply chain. More on why technology is used for data analysis is further elaborated below.

It should be noted that all four uses of technology are on the management of 'data'. In most cases, technology cannot overcome the lack of appropriate and accurate data. Recall the well-known adage: 'garbage in equals garbage out'. It is also important to discuss why we need technology to conduct the four tasks identified above. Technology is used to measure and quantify the return on investment for traceability. This includes identifying the benefits of implementing strong traceability systems for an individual company, product, supply chain, or food sector. Operational benefits include more visibility of a supply chain allowing for a leaner and more streamlined inventory control system. Technology can also be used to introduce confidence measures on the overall food safety and quality of a product line; such assurance metrics can then be used to instill trust in the consumer's perception of the brand. Technology, specifically data analytics, also proves invaluable during outbreak investigations by either narrowing the scope of the problem or by eliminating a supply chain from suspicion. Finally, traceability data can be mined by technology to create niche marketing advantages to reach unique consumer demands. Examples of such markets include claims of organic, sustainable, and environmentally friendly production.

5.9   Steps to achieve a global, traceable supply chain

Despite the recognition of traceability in the international arena (Codex setting the standard for traceability regulations and GFSI identifying requirements for industry practice), we are a long way from a fully traceable supply chain.

There are several ways to architect a traceability system (Meuwissen *et al.*, 2003; Bhatt *et al.*, 2012). When traceability regulations are in effect, they generally require one-forward, one-back traceability, meaning that a facility needs to record where the incoming product came from as well as recipients of finished products. During a food safety event, this means that information is obtained by going from one point in the supply chain to the next. There are a few other ways that traceability systems can be constructed. Some governments require the use of a centralized (or distributed) database for information. The greatest application of this system seems to relate to animal identification and registration. However, there are also numerous third-party technology providers who wish to 'connect the dots' throughout the supply chain. These providers accomplish this by having each supply chain member submit data to the third party. A third way to achieve traceability is to create a rolling record of information that accompanies the product. The term 'pedigree' means different things to different people, and is used differently from one industry to another. It is generally accepted that in a 'pedigree' system the full history of a product is available as a single record. The number of ingredients in food products, and the number of transformations, stages, and locations experienced by products (and their ingredients) before consumption, makes the thought of a food pedigree system overwhelming. However, technology may enable the likelihood of success. Whatever the architecture, there are several prerequisites to a good product tracing system and records must be kept by each supply chain partner that allow the product to be accurately traced.

Efforts to enhance the ability to trace products worldwide have been driven by private rather than government initiatives. The success of these efforts to trace products has been limited, primarily because they are voluntary and a truly effective system requires participation by all members. The patchwork of expectations of the regulatory authorities in different countries combined with the inconsistent enforcement of existing laws suggests that it will be some time before universal product tracing capabilities are demonstrated.

5.10 Summary and outlook

Traceability of food is a complex, multifaceted issue. Because cost is difficult to calculate and benefits are difficult to assign, some have argued that the government is best positioned to address traceability issues. However, most governments are focusing attention on preventive measures, hoping to avoid the food safety issues that trigger the need for traceability.

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6

Linking Local Suppliers to Global Food Markets: A Critical Analysis of Food Safety Issues in Developing Countries

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Summary

Food safety issues are gaining momentum across the globe, triggered by rising consumer awareness, technological up-gradation and with growing international trade. Initially, this chapter describes the development of global food supply chains and opportunities for developing countries in the global market regime. Food quality, safety and labelling issues are explored along with their implication on global trade and on the livelihood of farmers concerned. The role of local governments, international institutions (WTO, ISO, Codex Alimentarius) alongside the rise of public as well as private standards in food safety are highlighted. The roles of stakeholder collaboration, capacity building, corporate involvement etc., in the development of safe and efficient food chains are discussed. Case studies from selected agriculturally resource-rich countries are included. Finally, we provide important policy-level recommendations required to strengthen food safety standards in developing countries within the context of rapid globalization and a growing food trade.

6.1 Introduction

Food supply chains are undergoing rapid transformation in developing countries and a paradigm shift is underway for local and unorganized food supply chains from developing countries towards their integration with global food supply chains. This offers many market opportunities for developing countries in terms of food exports and generates livelihood opportunities, especially for small producers. However, there are many challenges related to food quality and safety to be met before developing countries can fully harness this potential. Food safety is also gaining momentum at the domestic front in developing countries, where the rising income of consumers provides scope for the food industry; at the same time, awareness levels and increased media attention pose challenges related to food quality and safety. The public sector has a prominent role in terms of food safety regulation. Food safety standards must be upgraded in these countries through policy-level changes related to food labelling, stakeholder collaboration, the role of the public sector, food safety regulation and risk assessment at the supply chain.

This chapter discusses several topics, including: (1) the development of global food supply chains; (2) opportunities for developing countries; (3) food quality, safety and labelling issues; (4) the role of the public sector and policy-related issues; (5) the role of international institutions (WTO, ISO, Codex Alimentarius); and (6) other issues such as stakeholder collaboration and capacity building. Case studies from selected agriculturally resource-rich countries are provided. The chapter also aims to provide important policy-level recommendations in order to strengthen food safety standards for developing countries within the context of rapid globalization and the expanding food trade.

With rising consumer awareness backed by technological changes, food safety issues now occupy a prominent role all across the globe. Multiple handling across different time zones and climatic conditions means that spoilage and/or contamination is more prevalent among foods which are exported across the globe. Improper storage and contamination makes food unfit for consumption and increases food wastage, as it is likely to fail phytosanitary standards. Concerns for food safety continue despite numerous technological improvements in the production and handling of food in different countries. The continuously changing nature of foodborne diseases and the discovery of new foodborne infections makes the handling of these issues more challenging.

The US-based Center for Disease Control and prevention (CDC) statistics reveal that the number of foodborne disease outbreaks resulting from imported foods has risen, especially in 2009 and 2010. Their review of foods imported in the US from 15 nations during 2005–2010 revealed there were 39 outbreaks and 2348 illnesses (Cook, 2012). Nearly 50% of these outbreaks occurred in 2009 and 2010, with 45% of these originating in food imported from Asia (Cook, 2012).

Foodborne diseases or illnesses can be caused by pathogens such as *E. coli*, food additives, contaminants, toxins, drugs or pesticide residue resulting in chemical poisoning. Lack of proper hygiene during production, packing, processing and transportation may lead to food-safety-related problems. It is also very difficult to maintain food safety standards in developing countries because of the fragmented and small-scale nature of the backward end of the chain (Cook, 2012). This requires a greater level of policy support in terms of standards and protocols and the accompanying regulation and enforcement of these standards. Appropriate training and development of all actors across the supply chain is necessary, especially at the critical points where food is most likely to become contaminated. At the same time, infrastructural support in terms of both primary and secondary infrastructure is needed to implement these standards in the field (Unnevehr and Hirschhorn, 2001).

Lack of safety standards may lead to rejection or cancellation of food imports from these countries, incurring huge losses by farmers and leading them to discontinue these operations as they are unable to cope with the situation. It is therefore imperative that raising the quality of standards fetches increased revenues and also

aids in improving the food safety and health of people domestically where food safety issues are already a threat (Unnevehr and Hirschhorn, 2001; Joshi, 2012).

6.2 The rise of global supply chains

Advances in transportation and telecommunications infrastructure have facilitated international trade, especially post-World War II. International trade has also benefited from the creation of an international institutional framework for international monetary policy, commerce and finance. To govern these agreements, several international institutions were founded to facilitate trade and lower barriers such as General Agreement on Tariffs and Trade (GATT) and the World Trade Organization (WTO).

Liberalized international trade and foreign investment, aided by advanced technologies, led to an increase in food exports (Coley *et al.*, 2011) of both fresh produce as well as processed. In addition, innovation and advancement in agriculture during pre- and post-harvest operations has benefited this sector by providing facilities such as increase in shelf life, grading, standardization and cold chains (Alexander and Nicholls, 2006). It takes only a few days for the produce harvested in Kenya or India to reach the supermarket shelves in Europe and the US after having passed through the stages of harvesting, sorting, packaging and transportation.

These innovations have led to the rise of global food markets where a few countries have developed robust international markets for particular commodities and are enjoying comparative advantages. More foods such as fresh fruits, vegetables, seafood and processed foods are being imported from the developing world than ever before. Among these prominent food-producing and trading countries, China holds a good position accounting for 17–18% of global wheat production, 15% of coarse grain production and 29% of global paddy rice production. It also accounts for 39% of global end-of-season wheat stocks,

30–33% of global coarse grain stocks and 53% of global rice stocks.

India also exports many commodities to developed countries such as seafood, tea, coffee, fruits and vegetables, including mango and grapes. Around 26.8% of India's total seafood exports reach European Union markets (Sarris and Morrison, 2009). Interestingly, although Europe as a whole accounts for almost half of the world's imports of horticultural products, the major importers such as the EU and the US import only around 1% of horticulture products from India (Nanda *et al.*, 2008). Kenya has also become a major horticulture exporter to Europe; fruit, flower and vegetable exports are now the country's major hard currency earner. A total of 95% of fresh produce exports go to Europe; most of the suppliers have agreements with large supermarkets in Europe (Brown and Sander, 2007).

The increasing popularity of supermarkets with consumers in the developed world means that supermarkets control most food sales in the developed region and the food suppliers in the developing world. Their rapid global expansion has created unlimited opportunities for suppliers in developing nations to trade in open markets. This has resulted in more control falling within the hands of these giant retailers, especially related to food quality and safety issues.

6.3 Global trade opportunities for developing countries

In most developing countries, which enjoy the comparative advantage of low labour costs and seasonal production, agriculture is the prime economic activity. These strengths can be exploited to create new opportunities for producers of these countries in terms of poverty reduction and livelihood improvement.

Quite often however, global sourcing leads to intense competition among growers from different countries which have location as well as time-based advantages. High profit margins leads to greater production and hence the oversupply

of commodities in the market, resulting in falling prices. Subsequently, many farmers are forced out of business due to a combination of issues related to lower prices in export markets due to untimely availability, loss of quality during transportation and other food safety issues, among which issues of pesticide residue have gained prominence during recent years.

In Argentina, the changing of large tracts of farmland from assorted multi-crop farming of local food varieties to genetically engineered soya monoculture farming has led to environmental, social, economical and rural decline (Valente, 2003; GRAIN, 2010). What started as an export earner for debt payment ended up as a farming disaster, leading to a situation where Argentina is now importing food which it used to export (Valente, 2003). Indian grape producers also suffered during the recent crisis in the global market caused by oversupply and superior quality grapes from other countries. A few of the export consignments were also rejected due to the presence of residues of chlormequat chloride.

Most farmers in developing countries are small and marginal, so it sometimes becomes difficult for them to compete with large commercial agribusinesses unless they obtain support from governments in the form of technical and institutional arrangements. In many developing countries (like India), governments have launched initiatives to educate farmers and provide hand-holding support for the adoption of both pre-harvest and post-harvest technologies (Vorley and Fox, 2004). Developed countries also provide some support to these countries in terms of strengthening the supply chains by building capacity of stakeholders and development of specialized infrastructure through their respective development initiatives (Valente, 2003; GRAIN, 2010).

Civil society organizations such as non-profit entities and growers associations can assist by consolidating the farmers' positions in the food supply chain through initiatives such as the collective buying of agri-inputs (including credit) and collective marketing of produce, thereby reducing transaction costs

(Hellin, 2009; Markelova *et al.*, 2009). Further, members can devise their own set of rules to complement the existing rules. However, many farmers' organizations lack specialized skills, financial resources, infrastructure and marketing expertise (Hellin, 2009; Markelova *et al.*, 2009).

6.4   Food safety issues: traceability, certification, labelling and phytosanitary

With the facilitation of international trade due to encouraging policy regime, better communication and transportation structure, supply chains are now more global in nature, yet there are many challenges in terms of geographic differences which remain to be overcome for further encouragement of trade.

6.4.1   Traceability and certification

Food labelling issues have also gained importance, especially in developed countries. Consumers are more concerned about the credibility of both products and the processes which are used to manufacture the food than ever before. This has resulted in more efforts to build transparency across the chain and has also facilitated two-way information between producers and consumers. Labelling also helps producers to obtain a competitive advantage in these markets.

For example, certification in the case of organic food is necessary; in absence of certification, it is difficult to distinguish it from non-organic food. Food labelling also offers unique benefits in terms of brand building within the minds of consumers, especially in developed countries. Certification is a tool to communicate product benefits to the consumers effectively (Blythe, 2006; Kotler *et al.*, 2009; Ramaswamy and Namakumari, 2009). Certification by a third party enhances the credibility of food in the minds of customers and brings

authentication to producers, traders and other middlemen (Searle *et al.*, 2004; Santacoloma, 2007).

Third-party certification can be a boon for farmers since it allows product differentiation (Weber, 2011) in terms of both socially and environmentally responsible products (Oelofse *et al.*, 2010; Ruben and Zuniga, 2011). A proliferation of different labels creates opportunities for market segmentation according to varied conditions prevalent at individual farms (Ruben and Zuniga, 2011; Sutherland, 2011). Consequently, it is vital to match types of standards with specific categories of smallholder producers to maximize long-term sustainable and optimum profits (Ruben and Zuniga, 2011; Sutherland, 2011). The high costs of implementing these standards sometimes prevent the farmers from adopting these standards, however. The presence of alternative and relatively smaller food certification agencies and group certification (Weber, 2011; Ruben and Zuniga, 2011), or public–private partnerships (PPP; Poulton and Macartney, 2011), could prove beneficial in this case. The large food processing companies sourcing Palm oil have been blamed for the destruction of tropical forests (Crowley, 2008; Food Ingredients First, 2011). Consumer demand has forced major food companies and retailers in developed nations to source their supplies from Certified Sustainable Palm Oil (CSPO) producers (Crowley, 2008; Food Ingredients First, 2011).

6.4.2   Labelling

A label is a form of assurance to the consumer for a particular standard. Codex, an international food safety UN organization, has also addressed issues such as: country of origin; date of expiry; and name of product through its Codex Committee on Food Labelling. The committee also provided its recommendations for labelling foods derived from biotechnology.

However, individual countries advocate different positions on this issue. Standards of identity for many food products have been developed through Codex committees which help to facilitate

> ## Food safety threatens spice exports from India
>
> The world's biggest exporter of spice is India, with total exports valued at over USD 1.1 billion. India is however facing regular rejections of its spice consignments from Europe, Japan, Australia and US because of failures to address issues of food safety, traceability and sustainability in its spice exports. As the regulations differ across different importing nations, it is not easy to fulfill all the required standards. It appears that the spice board in India has not been able to keep itself updated to address the increasing consumer demand for traceability and food safety among western nations. Public intervention in infrastructural facilities in processing and testing is required for long-term sustenance of the trade (adapted from Joshi, 2012).

international trade. Codex also emphasizes a uniform system of inspection for food import and export inspection and certification. The establishment of a uniform certification system is aimed at easing the export process, which will result in equal or greater protection of consumers at a reduced cost.

Food safety standards have also played an important role in developing markets for organic food. Today, nearly 100 regional or national organic standards have been developed worldwide (Santacoloma, 2007). Several developing countries such as India have also formulated national regulations regarding organic production, processing, certification and trade. Different organic food certification exists in the market with different criteria and standards. Some are internationally accepted, required for exports to major organic food markets in the world such as North America and Europe. The International Federation of Organic Agriculture Movements (IFOAM), USDA organic and Demeter Ingram labels are universally accepted by overseas markets (Santacoloma, 2007).

6.4.3   Phytosanitary issues

Exports of fresh food products such as fruit and vegetables represent a good opportunity because these products have a high demand and fewer trade barriers than staple commodity agricultural exports. However, fresh food products are also more likely to encounter sanitary and phytosanitary (SPS) barriers to trade (Unnevehr and Hirschhorn, 2001). Delivering safe food to distant markets requires process controls throughout the production process. Mechanisms need to be in place to assure buyers and government regulators that such controls are effective. Producers in developing countries must be made aware of the standard rules and guidelines.

Since 1995, Codex has been referenced as the organization to provide scientific advice on food matters brought before WTO under the SPS committee dispute settlement process. Codex guidelines for tolerances for food additives, contaminants and pesticide residues harmonize country requirements and also facilitate international trade.

It is important to develop partnerships between the public and the private sector, to provide training and market information and need for infrastructure related investments (see box). For example, a World Bank project for export promotion in the Ivory Coast supported a fruit export project. It helped a private organization to develop a system for quality and safety assurance for fruit exports to the EU. USAID activities in Guatemala have supported snow pea exports by developing integrated pest management (IPM) to limit pesticide residues, helping to reduce rejections in the US (Unnevehr and Wirschhorn, 2001).

Private–public partnership: exporting asparagus from Peru

Peru is a good example of a developing food-exporting nation which secured its lost market by following Codex rules. In 1997, Peru export sales of its flagship food asparagus fell due to two cases of Botulism from its produce in Spain. This motivated exporters to improve safety standards, as they became aware of the vulnerability of their asparagus export markets to such an ad hoc incident. The government and industry worked hand in hand to improve safety standards across the entire food chain. In 1998, the Peruvian Commission for Export Promotion worked with the asparagus industry by convincing them to implement Codex code on food hygiene. Seeing an improvement in production, worker efficiency and demand from international customers for certified produce, the industry complied with the national standards. Subsequently, many exporters developed the skills and experience to embrace the stricter certifications such as Hazard Analysis and Critical Control Points (HACCP) and Europe Retailer produce working group Good Agricultural Practices (EurepGAP) protocols.

The improved standards have generated demand for Peruvian asparagus, resulting in enhanced cultivation in newer areas and more employment opportunities. Peru has sustained its asparagus exports to the developed world because both the government and the industry developed and maintained national food safety standards in line with international norms (Jaffee and Henson, 2004).

6.5   Role of public standards

Food quality standards can be grouped into private and public standards. Public standards are governed by law and come under the purview of governments and international organizations. They can be enforced at the local, regional, national and even international level depending upon its mandate and its issuing authority. Private standards on the other hand cater more to their business interests with the customer as their prime focus. International multi-lateral organizations such as WTO, FAO and WHO have contributed and supported various standards with respect to food and its trade over the years.

6.5.1 Codex Alimentarius

Codex Alimentarius Commission was formed under the joint sponsorship of two United Nations (UN) organizations: the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) in 1962. Today, 165 countries worldwide are members of Codex. Codex Alimentarius Commission has two goals: to protect the health of consumers and to promote fair practices in the food trade. These goals are accomplished through development of food standards, food guidelines and codes of hygienic practices (WHO/FAO, 1999; Kimbrell, 2000; WHO, 2002; Codex Alimenterius, 2013).

Codex Alimentarius consists of several different types of committees that make recommendations. Committees include horizontal committees, commodity committees and regional coordinating committees. The horizontal committees are those whose mandate covers many commodities, and include Codex committees on food additives and contaminants, pesticide residues, veterinary drugs, food hygiene, fish and fish products, milk and milk products and food labelling. The Codex regional coordinating committees assist in the development of commodity standards that may be relevant at regional level. Further, they offer opportunities for regional members to develop strategies that may benefit them in other Codex committees. At the regional meetings, developing countries are able to participate and gather information about Codex activities in their region (Kimbrell, 2000; WHO, 2002; Codex Alimenterius, 2013).

Private sector organizations, including consumer groups and industry representatives, may join Government delegations at their discretion. The private sector may also attend meetings as part of a Codex-approved international non-governmental organization (INGO) and may offer comments during committee meetings (WHO/FAO, 1999; Kimbrell, 2000; WHO, 2002). It has been seen that public certifications such as the Codex Alimentarius protocol on food safety have benefited those producers who have been able to upgrade their standards; it can however sometimes be a barrier for small-scale producers and also prevent them from participating in international trade (Lee *et al.*, 2012).

Codex is in good agreement with the HACCP-based food hygiene system, which is a thorough preventive approach to food safety and addresses chemical, biological and allergenic hazards during production processes that may lead to an unsafe finished food product. It a preventive approach rather than post-preparation inspection, and can be used at all stages of the food supply chain.

6.5.2 Global Food Safety Initiative (GFSI)

Global Food Safety Initiative (GFSI) is a NGO created by Belgian law in 2000 (Cook, 2012). It is managed by the Consumer Goods Forum. GFSI evaluates existing food standards against food safety criteria. It builds consumer awareness on food safety, reviews current best retail practices and aids in information exchange along the supply chain. Various processes in relation to various commercial food safety programs are published in its GFSI Guidance document (Cook, 2012).

By 2012, eight major food retailers (Carrefour, Tesco, ICA, Metro, Migros, Ahold, Walmart and Delhaize) had agreed to the GFSI benchmark of food safety standards. The objective of this alliance is to make food production and processing as safe as possible. GFSI also runs a Global Markets Capacity Building Programme to involve the small and marginal producers and assist them in the development of food safety systems (Cook, 2012).

6.5.3 Food safety initiatives: Philippines

Food safety issues which have gained prominence in Philippines are those related to pesticide residues, toxins, allergy and physical and microbial contamination. The Philippine Food Safety System is led by two agencies: (1) the Bureau of Food and Drugs (BFAD) at the Department of Health (DOH) and (2) the Department of Agriculture. The regulatory tool used by BFAD is current

good manufacturing practices (cGMP) certification for all food industries. Additionally, the HACCP system is used to address food safety through the analysis and control of biological, chemical and physical hazards in the entire food chain from farm to table. The Department of Agriculture has several agencies: the Bureau of Animal Industry (BAI) for animal health; the Bureau of Plant Industry (BPI) for plant health, sugar, grains and milk; the Bureau of Fisheries and Aquatic Resources (BFAR) for the protection and safety of fisheries and aquatic resources; and the Fertilizer and Pesticide Authority (FPA) for the proper and safe use of pesticides.

6.5.4   Strengthening food safety initiatives: India

In 2006, India established its Food Safety and Standards Authority of India (FSSAI) under Food Safety and Standards Act, 2006. The agency is empowered to handle all food-related issues by consolidating the various acts and orders that were earlier handled in various ministries and departments. Its mandate is to create science-based standards for articles of food. It will also regulate the manufacture, storage, distribution, sale and import of food to ensure availability of safe and wholesome food for human consumption (FSSAI, 2013). The establishment of the Food Safety Act marked the abolition of previous systems. Various central acts such as: the Vegetable Oil Products (Control) Order, 1947; the Prevention of Food Adulteration Act, 1954; the Fruit Products Order, 1955; the Edible Oils Packaging (Regulation) Order, 1988; and the Milk and Milk Products Order, 1992 previously operated from different departments and ministries, making them cumbersome to implement effectively.

The existing act establishes a single reference point for food safety issues by changing from multi-level and multi-departmental control to a single-window system. This act is under the Ministry of Health and Family Welfare, Government of India. It enforces various provisions of the act along with state food safety authorities (FSSAI, 2013). It is mandated for standards of food products, standards for proprietary foods and health supplements, setting residue limits for pesticides and antibiotics in food products and the establishment of a surveillance network to test food articles for contamination, adulteration and false labelling. The act also aims to generate consumer awareness programs and provide training to people in the food business (Sharma, 2012a, b). In addition, it contributes to the development of international technical standards for food, sanitary and phytosanitary standards, important for exporters (FSSAI, 2013).

It recently launched a registration and license schemes for food safety for every business connected with food, including not only large organizations but also small road-side vendors. Businesses with an annual turnover below USD 20,000 will have to pay an annual registration fee of approximately USD 1.6. Those with a higher turnover will have to obtain a license ranging in price USD 30–120. The idea is to limit food outbreaks by improving hygiene.

6.6   Role of private standards in food supply chains

The key factors driving the role of private standards in global food value chains are increasing consumer interests in food safety and quality issues, rising regional regulatory requirements, the need for uniformity and quality across supply chains and privatization. All these factors related to consumer and regulatory pressures force the business to adopt stringent quality measures and, at the same time, protect their markets and brands. The role of private standards is growing across the globe with substantial impacts along the agri-food chain arising from the introduction of certification-based private food safety standards. To implement these private standards, shared value practices require to be created across the supply

chain where a wide number of stakeholders play a role, including multinational corporations who mainly source raw materials from developing countries.

Private standards help multinational corporations acquire new markets and build their brand image. The role of the standards is to facilitate the coordination among producers and the corporations by facilitating information flow and incorporating new products and technologies. Through these standards, there is a constant effort to control the quality of products and the conditions under which they are produced, processed and marketed. The role of voluntary standards is also to provide assurance on food safety, quality and risk management. Private standards appear to be more stringent.

Private standards for food supply chains also provide assurance that the food fulfils the requirements laid down by public standards (Jaffee and Henson, 2004). In fact, they are a step ahead of public standards; the latter only provide parameters for quality whereas private standards elaborate the operations and processes to comply with those parameters.

Private standards also set a higher standard for particular food product attributes and supplement the end-product food safety standards laid down by legislation. In addition, these standards fulfil the criteria as demanded by the importer related to ethical trade, environmental impact and social accountability. Similarly, the 'Field to Fork' standard of Marks and Spencer in the UK includes requirements that 'ban' around 70 pesticides in fruit and vegetables to be sold fresh or to be used as ingredients in prepared foods that are manufactured for sale under the Marks and Spencer own label. The view of private standards as more stringent or more extensive than public standards is probably the most widely held perspective on the relationship between private and public standards.

Private standards are further ahead in terms of achieving control over the supply chains in both vertical and horizontal directions. Increased vertical coverage refers to how far along the value chain controls are in place. Private standards not only pay attention to food safety and quality, but also see that the product and processes are followed in the supply chains which have social and environmental impact. In the case of exports, as well as fulfilling regulatory standards exporters adhere to ISO 14000 and SA8000.

Multinational companies source a large quantity of their products from small farmers and producers in developing countries. Implementation of these private standards therefore means working at the level of these producers. Most of the time, these producers are unorganized and lack the resources and expertise to implement these standards, as they require change in the production practices and processes related to these.

There are constraints in the system, however. Small producers lack the financial resources to upgrade their processes in order to follow the standards e.g., a standard may require the adoption of a next-generation pesticide, increasing the cost of production. Farmers may also lack expertise in shifting to new processes, thereby requiring extensive hand-holding support in terms of capacity building, training and implementation.

Individual certification may be much more expensive to farmers as compared to group certification. Small producers not only need to be educated on the methods/processes of production but also packaging, processing and transportation. Taking the apple supply chain in India as an example, much development has taken place in terms of using Good Agricultural Practices (GAP), modern packaging techniques and transport. Following the standards have in fact helped to reduce postharvest losses.

Adhering to private standards also means following safety protocols, documentation and internal and external auditing, which is laborious for small producers. The cost of implementing these standards, related to changing farming practices, training and capacity building, investment in control systems and infrastructure and certification costs are all high.

Graffham *et al.* (2007) calculated the cost of various schemes introduced by exporters to meet the EurepGAP standards in the period before its introduction in Kenya in January 2005; the cost per farm of different schemes ranged from USD 165 per farm to USD 4600. Multinational corporations outsourcing from developing countries benefit from not only sourcing quality products but also investing in the development of supply chains through infrastructure, training and capacity building.

The mandatory standards that are being implemented worldwide by the respective governments through regulation, social and environmental voluntary standards also involve certification and consumer labelling. Some of the key voluntary standards in the agri-food sector include International Foundation for Organic Agriculture (IFOAM), Good Agricultural Practices (global GAP), fair trade labelling, sustainable agriculture certification (Rainforest Alliance/Sustainable Agriculture Network), Roundtable on Responsible Soy (RTRS), Certified Sustainable Palm Oil (CSPO, created by Roundtable on Sustainable Palm Oil or RSPO), and sustainable coffee production (UTZ). Prominent voluntary measures in textile and clothing sectors that encompasses sustainable production include eco-labels such as Global Organic Textile Standard (GOTS), EU Flower and Oeko Tex 100.

Private certification standards have expanded on the premise that food and agriculture firms also want stricter norms to be adopted and want their produce differentiated from other available commodities. Despite being voluntary, these standards are gaining more importance throughout the world (World Bank, 2005; Hammoudi *et al.*, 2009; McCluskey and Winfree, 2009). EurepGAP, developed by 13 European retailers, is a type of private standard which attempts to link third-world farmers to international retailers (Lee *et al.*, 2012). It has expanded the scope by broadening the definition of food safety from pesticide use and residue limits to include hygiene and traceability requirements. (Lee *et al.* 2012). Associations or cooperatives can also be beneficial in creating a symbiotic relationship (Alonso, 2010, 2011; Weber, 2011).

6.7   Challenges faced by developing countries in food safety implementation

The implementation of food safety issues across the agricultural supply chain brings many benefits such as market access and premium prices paid to developing country producers, yet working at the field level with resource-poor and knowledge-deficient small and marginal farmers is a daunting task. This is further constrained by factors such as small landholdings, lack of awareness about safety standards, lack of specialized technical workforce and also weak market infrastructure and linkages. A strong policy, regulatory and institutional back-up is required to strengthen the food safety framework across the supply chains in these countries. In order to harness the global markets, developing countries need to consider many issues.

The logistics systems in developing countries are extremely poor, affecting both quantity and quality of the fresh produce and rendering much unavailable. The poor primary infrastructure such as roads, airways and trains is sometimes a hindrance when bringing the produce from remote areas to the mainstream markets. There is also a lack of specialized infrastructure such as grading and standardization machinery, storage warehouses, refrigeration infrastructure and ripening chambers. An effective and reliable cold chain system in developing countries such as India could play a role in reducing wastage (from local to national level), thereby strengthening the agriculture sector in India and improving food supply chain management (Joshi *et al.*, 2009).

6.7.1   Development of cold chains in India

It is estimated that there is a value loss of up to 40% in the horticultural produce supply chains in India. As the horticultural produce has to be transported over significant distances and climactic regions, the different environments affect the quality of the produce. A cold chain becomes

essential in developing a fresh produce supply chain. Due to the rise of the middle class, the demand for fresh and safe fruits and vegetables in the country is growing exponentially; the cold chain industry therefore has a critical role to play.

The size of the Indian cold chain industry is estimated to be nearly USD 2.8 billion per annum. It can play an important role in enhancing food safety standards, reducing post-harvest losses and increasing farmer incomes' and consumer wellbeing, thereby enriching the country, farmers and the consumers. The consumers benefit as these cold chains are expected to meet all the food safety standards through washing and reduction of chemical residues; fruits are therefore safer than those available through alternative channels. All other permitted post-harvest management practices are followed and the quality of the fruits and vegetables is maintained by following HACCP and GAP standards. The quality is ensured as the temperature across the chain is maintained until it reaches the consumer.

Food safety issues can be neglected in supply chains as there is a general lack of awareness about these issues among a majority of stakeholders (farmers, exporters and pack houses) regarding quality-oriented pre-harvest and post-harvest operations. Less-educated intermediaries with limited or negligible knowledge in quality also aggravate the problems.

Sometimes even exporters are unaware of the standards prevailing in the importing countries, hence are unable to add any value across the chains. The non-availability of modern packaging facilities to developing country farmers is also one of the major hurdles in food safety. In addition, since most farmers in third-world countries (including India) have small landholdings and smaller earnings, it becomes very difficult for them to upgrade to modern facilities without public or private sector investment.

In developing countries, production and pre-harvest operations are mainly traditional. Failure to follow pesticide regulations can result in the rejection of export consignments, leaving farmers to bear great losses. There are other issues related to packing materials used to maintain the quality of the food throughout the chain.

Public institutions play an important role in promoting awareness among farmers regarding food safety issues across the supply chains. Developing countries need such institutions which can establish linkages with the stakeholders but also connect with importing nations to develop commodity-specific standards. These institutions may also foster R&D in both pre-harvest and post-harvest operations. One role these institutions could play is that of building awareness about international food safety standards and also the capacity building of stakeholders. Further, development of secondary and specialized infrastructure such as post-harvest machinery including grading, standardization and packing are among the initiatives these institutions can provide. Another important task which these institutions can play at the national level is the development of food safety standards and protocols.

For example, the government of India has set up APEDA (Agricultural and Processed Food Exports Development Authority), the national level agency to connect the farmers to export markets. As well as promotion and branding and trade development, objectives include the efficient monitoring of pesticide residues and other agriculture chemicals in fresh foods for export to other countries and ensuring compliance with food sanitation laws. APEDA is working with many commodities including grapes, oranges and vegetables, but the promotion of mango exports through better pre-harvest and post-harvest practices and ensuring food safety is worth mentioning here. APEDA is taking various measures by defining the role of various stakeholders in the mango supply chain in India, as described in Table 6.1.

6.8 Conclusions and future outlook

Food safety issues are gaining attention on both domestic and global fronts; developing nations therefore need to undertake measures to strengthen the food safety of both fresh produce as well as

Table 6.1   Strengthening Food Safety through stakeholder collaboration: a Case of Mango Exports from India. Source: APEDA (2009).

APEDA (Agricultural Processed and Exports Development Authority) is the central body which works towards facilitating agricultural exports from the country by promotion, capacity building of farmers etc. For development of mango supply chains, their strategic role is to ensure that the exportable produce is as per the standards and they closely monitor the residue levels of pesticide and other chemicals in the produce. APEDA also works for devising regulations and systems for safe and judicious use of pesticides in the mango orchards and identify the sources of pesticide residues are other objectives of this central body. APEDA undertakes the following roles for the export development of mango

- Registration of orchard growers and packing houses.
- Recognition of accredited residue testing laboratories and national referral laboratories.
- Organization of awareness programmes for farmers.
- Collection and dissemination of data pertaining to food safety standards/pesticide residue limits of mango importing countries.
- Recommending package of practices to farmers.
- Preparation of promotional material, reading material, video films and other audio-visual material.
- Training of farmers, exporters, packers and other stakeholders along with help of local agencies.
- Market intelligence and brand development of mangoes in international markets.
- Recommended pest management practices and pre-harvest intervals.
- Research and development for innovative packing material and mango varieties

Role of National Referral laboratory and Residue testing laboratories:

APEDA has set up a National Referral Laboratory and residue testing laboratories to get the export samples tested. The role of the agency is to ensure that the exported mangoes pass the minimum residue limits as permissible by the importing countries. There is a National Referral laboratory set up under APEDA which undertakes withdrawal and testing a minimum of 5% samples directly from registered packing house facility. The samples already tested under accredited testing laboratories are retested at national referral laboratory. The NRL is also involved in training of analysts and also standardization of testing protocols for adoption accredited testing laboratories recognised by APEDA. The role of residue testing laboratory is to draw the representative samples of fruits for residue analysis in accordance with prescribed sampling procedures and ensure proper storage of samples, while testing and undertake testing of samples for residue determination in accordance with testing protocols specified in this residue monitoring plan. After this is done, the certificates are issued to exporters. The reports of all these tested samples are also made available through internet enabled software. These labs also adopt good laboratory practices throughout testing and maintain appropriate records for tracing back of samples to specific orchard.

Role of Registered Orchard Growers

The role of registered Orchard Growers are to follow the recommended pest management practices and pre-harvest intervals in the mango orchards for control of pests, to maintain the record of all field application of pesticides and any other agrochemicals in the mango orchards and monitor the incidence of fruit fly pests through pheromone (methyl euginol/cue lures) traps. These growers are also to permit the state agriculture/horticulture officers to undertake pre-harvest inspection of orchards to assess the incidence of pests and diseases and advise appropriate control measures and also to ensure that the appropriate samples are taken by the representatives of accredited testing laboratories

Role of Registered Exporters

The registered exporters are also advised not to use any misbranded or un-registered or banned or un-recommended pesticides in the orchards and also to adopt eco-friendly integrated pest management practices in the mango orchard to minimize pesticide residues. The role of registered exporters is to facilitate the interaction between growers and APEDA and also to verify that all the safety protocols are followed. The registered exporters main role is not to export fresh fruits from orchards/packing house facility not conforming with MRLs prescribed

(Continued)

under Food Sanitation Law of importing countries. They also establish SOPs for post-harvest processing including vapour-heat treatment of mangoes for export and verify that the recommended pest management practices and the pre-harvest intervals are adopted by the registered orchard. They are not to undertake any post-harvest fungicidal treatment of mango fruits for export and suspend the exports from that orchard in the event of failing of sample and to undertake further investigation into the cause of such failure. The registered exporters are also to source the fruits for export only from the registered orchards/packinghouse facilities and maintain all records related to export shipment-wise to facilitate tracing back of exports to specific packing house and orchard.

   The case study explains the role of a Government institution in a developing country like India to ensure the food safety throughout the supply chain. These countries where landholdings are small, farmers are unorganised and also lack technical know-how, such an agency could play an important role to strengthen the quality system across the chain. In case of mango supply chain, where the role of central agency as well as other stakeholders are defined, the system ensures that the safety norms are met, right material is exported, the capacities are built which ultimately leads to boosting international trade between developed and developing countries.

processed food. The prime motivation of developing a food safety programme in a country is to promote exports and empower the producers by creation of revenue generation opportunities. At the same time however, a food safety programme also benefits the nations in ensuring food safety on a domestic front. Considering the scale at which farmers and exporters are operating, it is not possible to ensure food safety without public intervention. Due to the large investment required to upgrade facilities to monitor and implement the program, intervention should be done in a phased manner starting with the area of the supply chain where the food is most vulnerable and at risk of contamination. Both policy-level and institutional-level measures are required to strengthen the food safety system in a country.

   The development of global trade brings opportunities for developing country farmers, so governments need to create a policy environment where trade is facilitated by strengthening food-safety-related infrastructure including upgrading food inspection capabilities along with sampling and laboratory facilities. There should be policy support in terms of upgrading secondary infrastructure pertaining to cold chains, grading and standardization facilities and ripening chambers. Primary infrastructure in the form of transport should also be developed in order to reduce transit time, helping to maintain the quality of food. The creation and development of new as well as upgraded existing food testing laboratories based on global standards will also help maintain food safety issues across the supply chain. The creation of human resources training opportunities is also important. The development of food safety protocols and their maintenance through controls and checks at various points and audits also must be ensured. Reviewing existing legislation and updating in line with the latest market and scientific knowledge pertaining to local food conditions and infrastructure is required to keep pace with international developments. Food legislation should ensure safe food supply internationally as well as domestically. Increased effective collaboration among food industry, relevant government agencies, civil society, consumer groups, academia and international organizations is also important in strengthening the system.

   Operational issues are also important as many food safety issues need to be implemented in the field. One of the major issues in this area is related to the awareness and capacity building of farmers. There must be rigorous efforts in organizing farmers at the local level in region-specific and commodity-specific groups in order to facilitate their training and capacity building. Farmers and other stakeholders in the supply chain also

must be made aware of the existing public and private standards and how these need to be met in order to gain better prices. Infrastructural and operational support must be provided in order to educate producers about the standards prevailing in other countries. If pursued at the local level, such initiatives will surely lead to multi-level development in the form of boosting international trade, capacity building at local level, empowering farmers and developing safe food chains across the globe.

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7
Achieving Quality Chemical Measurements in Foods

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Summary

Laboratory test results in food analysis are very important to the agro-food industry and public. The presence of inaccurate data for routine measurements of food ingredients, nutrients, contaminants and additives is wasteful of resources, could jeopardize legal decisions and poses threats to our public health and safety. As a consequence, international harmonization of quality assurance protocols has been developed for laboratories worldwide to ensure appropriate quality control is in place. The milestone in standardization of internationally accepted good laboratory practice is ISO/IEC17025 - General requirements for the competence of testing and calibration laboratories. The standard document highlights method validation, measurement uncertainty (MU) and various quality control procedures such as the essential requirements for laboratories to produce consistently valid results in chemical testing and calibration. In addition, metrology in chemistry (MiC) is a discipline concerning accurate chemical measurement through the implementation of traceability, comparability and MU. The establishment of a national MiC framework from recognizable reference standards or systems provides a crucial foundation to promote global recognition of analytical results. Standardization of laboratory quality assurance protocols and application of MiC are considered to be vital to ensure reliable analytical results in food measurement.

7.1   Introduction

The production of accurate experimental data is of paramount concern to the world of measurement science and it is also the ultimate target of analysts and their organizations. The presence of incorrect or misleading test results can hamper decision-making and lead to the ubiquitous loss of invaluable resources. A reliable measurement system ensures a sustainable national development in technology, health, environment and all societal aspects that relate to and rely upon data analysis and assessment. Confidence in measurement also gives rise to the advantage of accessing global markets and reduces unnecessary barriers to trade (Karrls, 2007). Food safety is today recognized to be a global issue that affects hundreds of millions of people. Attention has always been paid to improving the quality of analysis in food safety, which underpins national economy and worldwide stability (Karrls, 2006).

Over the past decades, we have experienced a number of food crises that have severely affected consumers and businesses. Recent food contamination cases include the presence of dioxins in human food and animal feed, Sudan dyes in chilli powder, melamine in dairy products and phthalates in beverages. All of these unfortunate events had a profound impact on the food industry and weakened the confidence of consumers. On the other hand, lack of adequate testing capabilities has inevitably led to unexpected and huge losses of resources and money as the following two examples show. The import ban of Nile perch to EU countries from Kenya in 1999 (Abila, 2003) due to the high levels of residual pesticides caused a catastrophic loss of about USD 35 million per year and the disappearance of many local jobs at that time. A six-month suspension of freshwater prawn from Bangladesh consignments in 2009 due to the detection of the metabolites of banned nitrofuran antibiotics by port health laboratories in the EU resulted in a loss of USD 350 million and the closure of many shrimp processing plants. The rejection of fishery products from these two countries shared common features in the exporting countries of inadequate laboratory facilities

and poor quality systems to achieve reliable measurements. To safeguard national interest, relevant authorities of both countries had no choice but to reinforce the measurement infrastructure to the international level as one of their major remedial actions. The two food incidents are obviously the tip of the iceberg, and such painful lessons may be repeated in the absence of appropriate food control management systems. Part of the management can be secured through legislation, effective enforcement and education of the food industry and consumers. A key feature is the implementation of quality control in the analysis of measurands in food products, however.

Quality measurement is the core of modern analytical chemistry and analytical services such as in food analysis. This is mandatory in some food sectors where legislative considerations or legal limits are involved, for instance, EU regulation on foodstuffs that have threats to health (European Commission, 1993) and on maximum residue levels (European Commission, 2005). Thanks to the tremendous commitment and cooperation of various organizations over the past two decades, a number of international protocols on standardization of analytical work in chemical laboratories have been issued as useful guidance for laboratory personnel (Thompson and Wood, 1995; OECD, 1998; Thompson *et al.*, 2002; CITAC/Eurachem, 2002). In general, these protocols provide harmonized recommendations to stakeholders to establish reliable measurements across different types of laboratory operations. The most notable document in enhancing the standardization of good laboratory practice in chemical analysis is ISO/IEC 17025 (ISO, 2005). Since its launch in 1999, the ISO/IEC 17025 standard has been extensively adopted and used as the key to quality control by chemical laboratories across the global. The standard specifically focuses on 'fitness for purpose' method validation, measurement uncertainty (MU), internal quality control and external quality assessment as well as an extensive range of relevant technical and management requirements. Laboratories should competently demonstrate that their analytical methods have undergone all

the required quality control procedures as stipulated in the ISO document.

Analytical results have limited value, and their associated uncertainty claimed will not be meaningful if the measurements are carried out without an agreed metrological traceability chain. The chain requires a pre-established calibration hierarchy which can be linked to an international or national reference standard or system. With a well-defined traceability in the measurement, the measured quantity can be compared (comparability) irrespective of when and where the analysis was performed. Metrology in chemistry (MiC) is a discipline concerning accurate measurement in chemical testing through the detailed understanding of an analytical method. Traceability, comparability and MU are key concepts in MiC and the application of these metrological concepts is specifically important in food measurements (Iyengar, 2007). In fact, the National Metrology Institutes (NMI) and Designated Institutes (DI) are responsible for establishing the corresponding national measurement standards or systems under the Metre Convention to ensure effective dissemination mechanism of MiC to their field laboratories in the analysis of food and other sectors (Kaarls, 2006).

This chapter aims to highlight the importance of quality assurance concepts in ISO/IEC17025 and discusses the infrastructure and development of MiC concerning reliable measurement in food analysis. Proficiency testing programmes using assigned reference values for melamine in milk and pesticides in green tea are used as case studies to illustrate the actual difficulties in the determination of trace levels of organic contaminants in food matrices by food testing laboratories.

7.2   Quality assurance in food analysis

The two principal sections in ISO/IEC 17025 cover the management and technical requirements in calibration and testing work. Management requirements address the operation and effectiveness of the quality management system within the laboratory. Items relating to organization and management, the quality system, document control, contract review, tendering and purchasing services, complaints, control of non-conforming work, corrective and preventive actions, records, internal audits and management reviews are described in detail in the document. Technical requirements determine the correctness and reliability of the tests and calibrations performed in the laboratory through the method validation process and staff training. Figure 7.1 shows the schematic quality control system implemented in a food testing laboratory as stipulated in ISO/IEC 17025. The areas on calibration with defined traceability, used of certified reference materials and the participation in proficiency testing programmes are the important quality tools to reduce measurement bias.

7.2.1   Method validation

Method validation is an essential requirement in the practice of any of the analytical methods which form the fundamental level of quality assurance in laboratories (Olivares and Lopes, 2012). A thorough validation process provides information about the analytical bias of the method within a statistical estimation of confidence. ISO/IEC 17025 specifies that validation must be applied for all analytical methods developed and each method should be validated such that it is suitable for its intended use, or fit for its purpose. Furthermore, analytical chemists should always exercise scientific knowledge and logical good sense prior to the commencement of method validation. Some common considerations should be included, such as (but not limited to):

1. understand the type of analytes and matrices to be analysed;
2. select the most suitable instrumentation available in their laboratories for the analytical method and ensure the instrumentation is in good condition and optimized for the analyte and matrix;
3. take adequate aliquot weights of samples for replicate analyses from a well-homogenized sample;
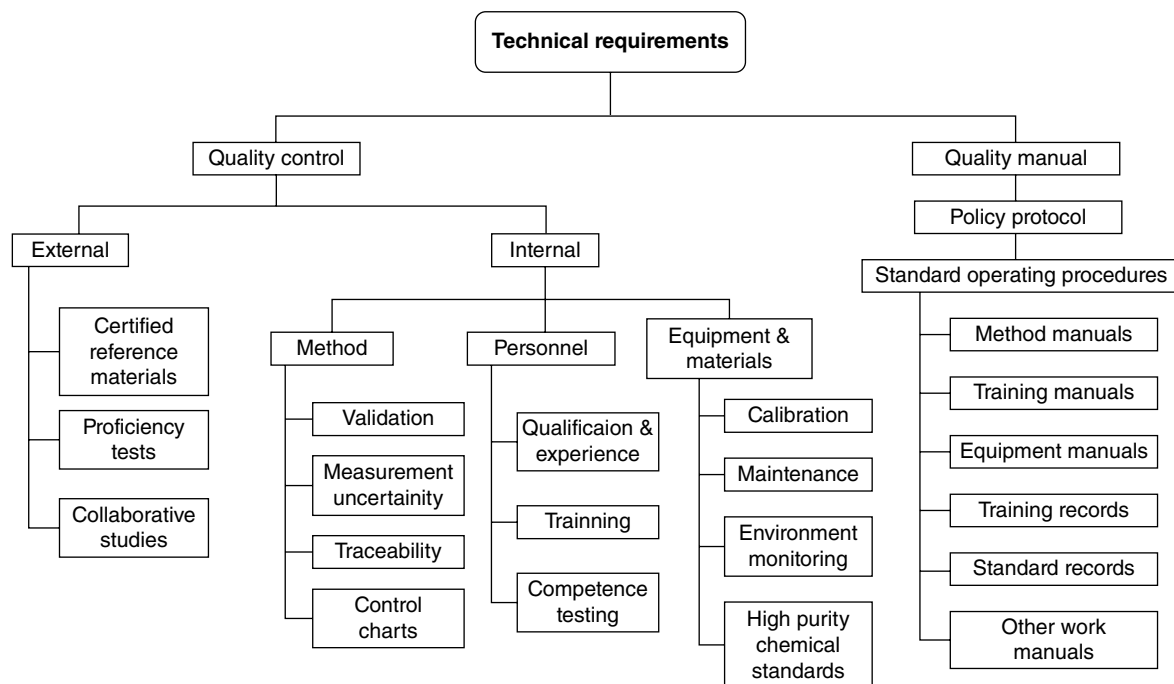
Figure 7.1   The technical requirements for competent food laboratories outlined in ISO/IEC 17025.

4. use high-purity solvents and chemical standards;
5. ensure a good working environment to avoid contamination during analysis and storage (temperature and humidity are controlled whenever possible); and
6. prevent careless errors in standard preparations, data transferring and calculation.

ISO/IEC 17025 provides the conceptual framework for quality measurements in chemical laboratories, but no in-depth descriptions and elaborations of how the process is to be carried out. Because of that, several international organizations have published guidelines (FAO, 1997; AOAC, 2002; CITAC/Eurachem, 2002; EURL, 2011) that purposely outline the necessary technical components that need to be taken into account and performed during method validation of quantitative analysis in food (Table 7.1). The guidelines have slightly different approaches, but all have the universal goal in method validation that the performance characteristics should

be established to prove the method is suitable for its intended used.

Since method validation work is costly and labour intensive, the extent of each validation process should be based on the laboratory's ability and requirements and take into account the customers' needs. The above guidelines and the literature (Araujo, 2009; Stöckl et al. 2009) describe typical basic validation as evaluating key performance characteristics of an analytical method including selectivity, linearity and range, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and ruggedness.

Selectivity represents the response(s) to an analyte or a number of analytes under given conditions so that the analyts(s) can be determined accurately in the presence of chemically similar substances or interference substances. Selectivity and specificity are used interchangeably in the literature, but an IUPAC technical paper (Vessman et al. 2001) reported that selectivity is the most suitable term to be used in analytical chemistry. A selective method is based on

Table 7.1   Method validation for quantitative analysis in food outlined by various documents.

Document title	Reference	Recommended performance parameters
Validation of Analytical Methods for Food Control	FAO (1997)	(a) Specificity: analysis of a set of representative blank samples (b) Accuracy: at or below MRL should be greater than those at or above MRL (c) Precision: method's repeatability and within-laboratory reproducibility (d) Limit of quantification: the smallest measured content with a specified degree of accuracy and repeatability (e) Sensitivity: slope of analytical calibration (f) Practicability and applicability: apply to broad range of matrices and analytes
AOAC Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals	AOAC (2002)	(a) Applicability: demonstrate acceptable recovery and repeatability with representative matrices and concentrations to which it is intended to be applied. A matrix blank is required for low-level analytes determination (b) Selectivity: test in the presence of accompanying analytes or matrices most likely to interfere (c) Calibration: fit a signal response against the concentration containing 6– 8 points, approximately equally spaced over the concentration range of interest. A high correlation coefficient (e.g. >0.99) is often recommended as evidence of goodness of fit. (d) Reliability characteristics: accuracy, repeatability precision, measurement uncertainty, reproducibility precision, intermediate precision, limit of determination (e) Limit of quantification: usually the lowest point on the calibration curve (f) Control of analytical data: control chart, injection control to check instrument stability and duplicate control (repeatability) (g) Confirmation of analyte: using specific methods such as mass spectrometry (h) Stability: assay periodically for a period of time judged to reasonably exceed the shelf life of the product
Guide to Quality in Analytical Chemistry: An Aid to Accreditation	CITAC and Eurachem (2002)	(a) Sensitivity: ability to determine particular analyte(s) in complex matrices without interference (b) Range: good linearity using five different concentration levels including blank (c) Linearity: regression line against analyte using the least squares method (d) Limit of detection: correspond to 3 standard deviations of repeat analysis of blank (e) Limit of quantification: usually the lowest point on the calibration curve (f) Ruggedness: to test small changes deliberately introducing to the method and examining the consequences (g) Bias: using reference method of known and small bias; closely matched matrix CRM to study losses, contamination, interference and matrix effects (h) Precision: reproducibility of results from different operators, different laboratories, different equipment and during time period

(Continued)

Table 7.1   (Continued)

Document title	Reference	Recommended performance parameters
Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed	EURL (2011)	(a) Mean recovery: use spiked samples to check the accuracy of the method (b) Precision and sensitivity: a minimum of 5 replicates is required at both the reporting limit, and at least another higher level or an action level (c) Limit of quantification: the lowest validated spike level meeting the method performance acceptability criteria (mean recoveries in the range 70–120%, with an RSD ≤ 20%) (d) Inter-person variability: incorporates two or more analytes

optimized separation, purification and detection mechanisms. Appropriate sample pre-treatment such as liquid extraction, solid phase extraction and headspace solid phase micro-extraction could improve method selectivity. Furthermore, the state-of-the-art hyphenated mass spectrometry techniques such as liquid chromatography with tandem mass spectrometry (LC-MS/MS), LC with time-of-flight MS (LC-TOF/MS), gas chromatography with tandem mass spectrometry (GC-MS/MS), etc. allow (in experienced hands) unprecedented reliability in the identification of analytes. As a guide on method selectivity, the European Union (EC, 2002) introduced an identification point scale on the analysis of veterinary drug residues for the identification and confirmation of an illegal (Group A) and legal (Group B) analyte (Table 7.2). The EC Directive recommends a minimum of 4 and 3 identification points for Group A and B analytes respectively for quantification. It also reflects the superior selectivity of mass-spectrometric methods over non-mass-spectrometric methods.

Linearity of the target concentration range of the method should be examined and explicitly specified. A common technique is to construct a calibration curve by plotting the instrument response against a series (of at least 5 points) of

Table 7.2   The relationship between a range of classes of mass fragment and identification points earned

MS technique	Identification points earned per ion
Low-resolution mass spectrometry (LRMS)	1.0
LR-MS^n precursor ion	1.0
LR-MS^n transition products	1.5
High-resolution mass spectrometry (HRMS)	2.0
HR-MSn precursor ion	2.0
HR-MSn transition products	2.5

Notes: each ion may only be counted once; GC-MS using electron impact ionization is regarded as being a different technique to GC-MS using chemical ionization; different analytes can be used to increase the number of identification points only if the derivatives employ different reaction chemistries; HPLC coupled with full-scan diode array spectrophotometry (DAD); HPLC coupled with fluorescence detection; HPLC coupled to an immunogram; two-dimensional TLC coupled to spectrometric detection; a maximum of one identification point may be contributed, providing that the relevant criteria for these techniques are fulfilled; transition products include both daughter and granddaughter products

high-purity analyte standard solutions at different concentrations or analyte standards that are dispersed in a matrix similar to that of the test samples. The goodness-of-fit of the data to

the curve using linear regression gives information on the correlation coefficient (r^2), slope and y-intercept of the curve. The closer the r^2 value to 1, the better the linearity of the data points. In practice, an even distribution of calibration points is preferred to avoid a weighting effect of high-end points on the curve causing significant linear regression deviation. For nonlinear calibration curves, mathematical manipulation could be used to transform to linearity (e.g. logarithm scale).

Sometimes the standard addition method can be employed in complex sample matrices where the preparation of a simulated blank sample is difficult. The range of an analytical method is described as the interval between the upper and the lower calibration points that have known (determined) accuracy and precision.

Accuracy is the bias from the 'true' value and is defined as the closeness of agreement between a test result and the true value or the accepted reference value (ISO, 1994). The method could be verified with standard methods of known accuracy that have undergone multi-laboratory validation, but the use of matched matrix certified reference material (CRM) is the most practical alternative to determine method bias. CRM is a material sufficiently homogeneous and stable with respect to one or more specified quantities, accompanied by certified traceable property values. Its use for the calibration of an apparatus, assessment of a measurement method or assigning values to materials is well established (ISO, 2007). Taking into account the associated uncertainty, comparison of the mean of replicate analyses with the assigned reference value of CRM can give a good estimation of bias at the determined concentration level (which should be within the linear calibration range). Appropriate CRM with different concentrations should be necessary to express method bias over a particular concentration range. However, the supply of CRM with respect to the wide variety of analytical methods is very limited and accuracy

determination can be circumvented by laboratory reference materials (ISO, 2012; Amigo *et al.* 2004). The production of laboratory reference materials is still tedious and requires skilful characterization techniques; laboratories therefore often prefer to address the issue with in-house quality control or spiked samples, where known quantities of analytes are added to the blank and sample matrix. The bias, in terms of recovery, can be expressed as the difference between the determined and the nominal values. The recovery R is given by:

$$R = \left(m_{\text{total}} - m_{\text{native}} \right) / m_{\text{spike}}$$

where m_{total} and m_{native} are the masses of analyte found in the spiked and unspiked portions respectively, and m_{spike} is nominal mass of analyte added to the analyte-free portion (AMC, 2000).

Since spiked samples do not normally behave exactly the same way as that of incurred samples, good recovery does not necessarily guarantee a good accuracy; poor recovery is certainly an indication of significant inaccuracy in the spiking study, however (Thompson *et al.* 1999).

Precision is defined as the closeness of agreement between independent test results obtained under stipulated conditions (ISO, 1994). Precision is usually determined by the relative standard deviation of replicate analysis of CRM, spiked samples or test samples within the same day or different days (repeatability). The concentration should cover the expected linear range and selected concentrations in the study should include one close to the quantitation limit, one at the mid-point and one at the highest point of the calibration curve. Another precision study is to determine statistically the variance of duplicate analysis in a number of successive runs using one-way analysis of variance (ANOVA). Precision could also be assessed under different sets of conditions, for example different operators (reproducibility).
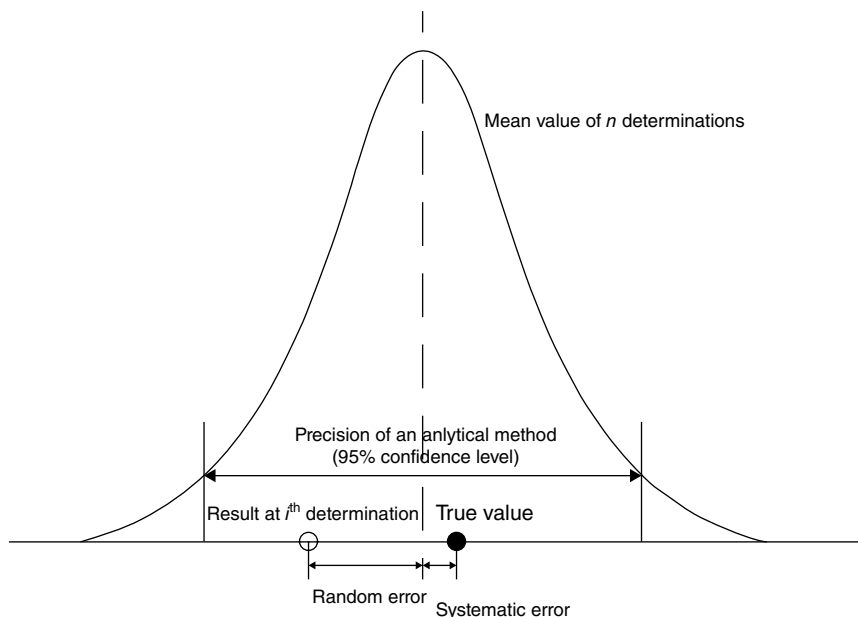
Figure 7.2   Probability of a measurement following a Gaussian distribution pattern due to random and systematic errors; 95% of the data fall within ±2 times the standard deviation from the mean value.

The presence of systematic errors and random errors in measurement governs the degree of accuracy and precision of a method, respectively, as illustrated in Figure 7.2. One of the objectives of method validation is to give a comprehensive evaluation of the errors present in the method. Acceptance criteria for individual performance parameters actually depend on the sample matrix, sample processing procedure, instrument used and analyte concentration, and are on a fitness-for-purpose basis. Although no specific require-ments are stated in the guidelines, general accept-able figures for the accuracy (recovery) and precision (repeatability) are recommended by the AOAC Single Laboratory Validation Protocol (Table 7.3).

LOD is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified. It can be estimated by visual inspec-tion of the detectable signal arising from the mini-mum amount of analyte. Theoretical approaches by taking three times the signal to noise ratio or by a use of a factor of the standard deviation of signals generated from a number of blank

Table 7.3   Recommended acceptance criteria for recovery and repeatability by AOAC (2002)

Concentration	Recovery (%)	Repeatability (%)
100%	98–101	1
10%	95–102	1.5
1%	92–105	2
0.1%	90–108	3
0.01%	85–110	4
10 μg g^{-1}	80–110	6
1 μg g g^{-1}	75–120	8
0.01 μg g g^{-1}	70–120	15

samples are also adopted. On the other hand, LOQ is the lowest concentration of analyte in a sample that can be quantified with given quality assurance. LOQ can be defined by signal to noise ratio (>10) or a factor (3–10 times of that of LOD) of the standard deviation from a number of blank samples, or the lowest concentration point in the calibration curve. No matter which is chosen, it should be checked by spiking the concentration into sample matrices to ensure the empirical value obtained is practically detectable.

Ruggedness is a measure of the quantitative influence on test results of a variety of conditions (variables) within a realistic range such as sample size, extraction solvent composition and volume, extraction duration, injection volume, mobile phase composition and its flow rate, column temperature and column type. AOAC recommends a Youden Ruggedness Trial (Youden and Steiner, 1975) in which the trial allows investigation of the effect of 7 parameters in a single experiment requiring only 8 determinations. The influence of each variable can be assessed by applying the student *t*-test. If the calculated *t*-value results are lower than the critical *t*-value, then the method is rugged with respect to that change of parameter in the procedure; otherwise, the method is not rugged for that parameter.

Finally, analytical chemists should be reminded that method validation must be carried out such that the performance of the method can be re-characterized whenever the conditions have been modified, for example, when an analytical instrument is newly installed or after a major instrument failure or breakdown occurs. Revalidation of the analytical method is also required upon any change of the performance characteristics, for example, to improve detection capability, include other matrices or use other extraction solvents.

7.2.2 Control chart

As stated in ISO/IEC 17025, 'The laboratory shall have quality control procedures for monitoring the validity of tests undertaken. The resulting data shall be recorded in such a way that trends are detectable and, where practicable, statistical techniques shall be applied to the reviewing of the results. Quality control data shall be analysed and, where they are found to be outside pre-defined criteria, planned action shall be taken to correct the problem and to prevent incorrect results from being reported.' That is, a continuous evaluation of the validated method should be implemented to address the quality requirement. The control chart is a versatile tool for quality control of daily analytical work and is based on the analysis of control materials during routine analysis. The values of the control material obtained in each batch of routine analysis are plotted in a concentration–time graph. Upon accumulation of sufficient data points, the control limits can be established to monitor the performance of the work. The construction of a control chart is based on the assumption of normal Gaussian distribution of data. The central line is the mean of the control samples collected over a specified time period. Since approximately 95% and 99.7% of data fall respectively within ±2 and ±3 times the standard deviation, respectively, warning limits (WL) and action limits (AL) are set at these values. Statistically, only 0.03% of normally distributed data are outside the AL, indicating that the probability of an erroneous measurement outside the AL is high. The benefit of using a control chart is an immediate and clear indication of the quality of process simply by observing its location on the chart.

As an illustrated example, Figure 7.3 depicts a CRM of aflatoxin B1 (certified value at 2.6 ± 0.4 µg kg^{-1}) in compound feedstuff is used as the control sample for the aflatoxin B1 analysis in an accredited laboratory. The mean value (central line) is at 2.48 µg kg^{-1} and is 0.25 µg kg^{-1}.

The analytical process is demonstrated to be in control because: (1) all control points are within the control limits; (2) the majority of the control points are near the central line and only a few are close to the control limits; and (3) about an equal proportion of control points are randomly distributed around (above and below) the central line. It is worth pointing out that 7 consecutive points occur from sample batches 8 to 14 on the chart. The overall process is in control, but may be considered to be going out of statistical control if 7 control values in consecutive order exhibit a gradual increase or decrease about the central line (Hovind *et al.* 2007). In this case, the analyst can report the results but should note that a problem may be developing and should be alert to avoid further deterioration of the process. On the contrary, the process is out of control if any control point is outside the AL, or the control point lies between the WL and the AL and at least one of the two previous control values is also in that region. Remedial action to investigate
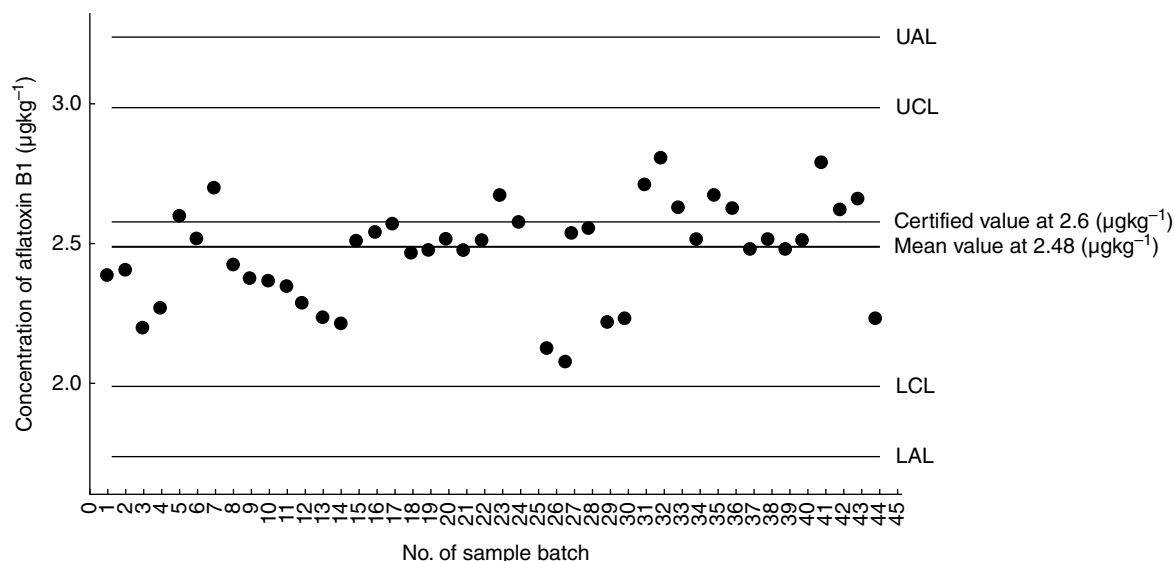
Figure 7.3   A control chart for monitoring the analysis of aflatoxin B1 in feeds. UAL, UCL, LAL and LCL represent the upper action limit, upper control limit, lower action limit and lower control limit, respectively.

the root cause should be taken and no analytical report should be issued.

Control charts are very useful if the control samples have similar properties to those of the test samples and can go through the entire analytical procedure. On some occasions, however, laboratories use blanks as the control samples for revealing the validity of the LOD and LOQ of the method. Furthermore, the use of standard solutions only provides repeatability information (random error) on the instruments, but shows little information on the accuracy of the method (systematic error). Quality control samples, spiked samples and real samples are therefore commonly used as the control materials in food laboratories as long as these materials are homogenized, consistently stable, are of sufficient quantity to be used for a long period of time and contain analytes within the linear concentration range.

Matrix CRM are expensive but are highly recommended if they are available. There is no restriction or rule on the number of control materials in each run. In view of containing the analytical cost, usually one control is used but the number can be increased if the analytical time is long, e.g. overnight operation. It is also preferably to have one at the beginning and another one at

the end of the experiment in order to accommodate the drifting of instrument response. Based on the spread of the accumulated control points, the control limits and central line could change with time and it is recommended to review these parameters at least once a year. Any update and revision or WL and AL in the control chart can be performed regularly on the basis of laboratory policy.

7.2.3   Traceability

Method validation not only demonstrates the ability of a method to measure analytes in specified matrices from its performance characteristics, but it is also a process to establish traceability through its calibration standards or system. Traceability requires a complete understanding and definition of the measurand and can ensure measurements performed in different laboratories can be meaningfully compared, or termed as comparability (King, 2000). The concept of 'tested once and accepted everywhere' can come into reality if measurement methods possess known traceability (Kaarls, 2007). Traceability is defined to be the property of a result of a measurement or the value of a standard whereby it can be related to stated references, usually national or

international standards, through an unbroken chain of comparisons and their associated uncertainties (ISO, 2007). Wherever possible, a traceability chain of measured values terminates in an SI unit. Unlike a physical measurement, maintaining a traceability link is challenging in chemical or food analysis as some of these measurements are method-dependent, such as dietary fibre analysis (Mertens, 2003), where the comparability of results is only made by strictly adhering to a particular analytical protocol. The sampling plan also requires attention in food analysis because the number, size, frequency and nature of the samples taken have significant influence on the final results. These are the primary sources of error and are one of the weakest links of the traceability chain (Quevauviller, 2004). The laboratory should make clear reference to their methods to appropriate international guidelines such that the traceability chain is not interrupted.

Furthermore, sample treatments such as derivatization, clean up and extraction that might be involved in the test procedures prior to the measurements must be referenced. These indirect determination procedures may undermine traceability if no suitable reference point can be made. The ISO definition is in coherence with those in the Eurachem/CITAC guide in this aspect. The guide (Eurachem/CITAC, 2003) describes how traceability should be established through the following steps during method development and validation: (1) specify the measurand, the scope of measurement and the required uncertainty; (2) choose the method of measurement; (3) validate the method of measurement; (4) identify/quantify all influences that will affect the results; (5) choose appropriate references; and (6) estimate the uncertainty components associated with all influences and references.

The measurement method must therefore be validated and standards and equipment used must be properly calibrated with a fully defined source of error. By doing so, traceability of the results from a validated method of known uncertainty is related to the traceability of reference standards or methods, which in turn is linked to the International System of Units

(the SI) via recognized national systems (BIPM, 2006; Barwick and Wood, 2010). Traceability of results can be established to link to more than one well-stated references or standards, and also the documented 'history' of a product or a system (Taverniers *et al.* 2004).

Examples of the traceability chains of two methods in food analysis in our laboratories are shown in Figures 7.4 and 7.5. In Figure 7.4, the analytical results of vitamin A (retinol) in infant
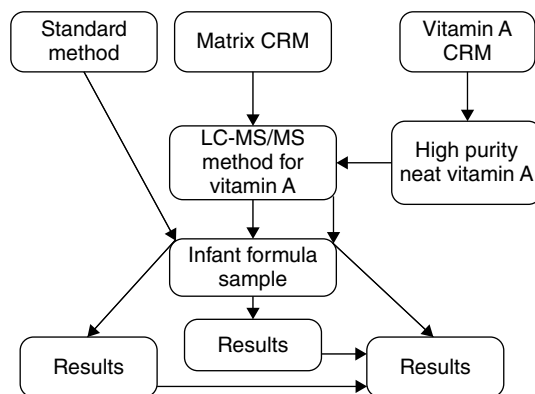
Figure 7.4 Results of vitamin A generated from a LC-MS/MS method are traceable to a standard method and a CRM through an unbroken chain.
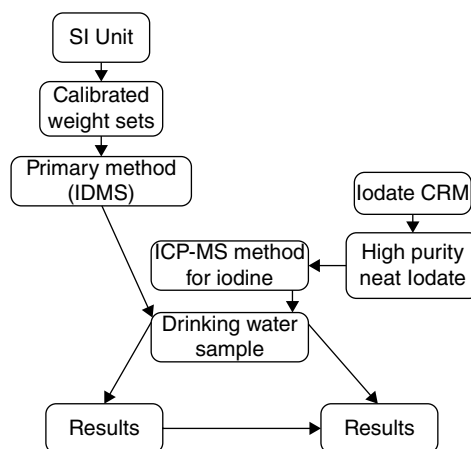
Figure 7.5 Results of iodine in drinking water generated from an ICP-MS method are traceable to the SI unit through an unbroken chain. The neat iodate standard is traceable to an iodate CRM.

formula by a validated LC-MS/MS method are traceable to the stated national standard (e.g. NIST SRM1846) and the standard reference methods (e.g. AOAC Official Method 992.06). The vitamin A CRM (e.g. USP standard) verified the purity of neat vitamin A, which in turn was used in method validation. In this case, the neat vitamin A used as the calibration standards also linked to the vitamin A CRM. Another example in Figure 7.5 illustrates that the ICP-MS results of iodine in drinking water are traceable to the SI via a primary method of gravimetrically isotope dilution mass spectrometry (IDMS) and the iodate CRM (e.g. NMIJ CRM3006-a).

7.2.4   Measurement uncertainty

Results obtained in repeated measurement of a sample using the same test method are characterized by a normal Gaussian distribution (Figure 7.2) due to the presence of random and systematic errors. The latter affects the bias from the true value (accuracy) and the former affects the repeatability and reproducibility of data (precision). All these error components contribute to the uncertainty of the test method. Estimation of MU in chemical measurement is one of the important pre-requites stipulated in ISO/IEC 17025 as the MU itself explicitly indicates the quality of the test results. MU is defined as a parameter associated with the results of measurement that characterizes

the dispersion of the values that could reasonably be attributed to the measurand (ISO, 2008). MU is scientifically useful to interpret or to judge the measurement results in order to make correct decisions in legal proceedings.

As illustrated in Figure 7.6, the residue amoxycillin in four porcine muscle samples A to D was obtained by a LC-MS/MS method that was characterized by a relatively expanded uncertainty of 20% at the 95% confidence level in the 20–80 μg kg^{-1} range. The results of sample A less the MU is above the MRL at 50 μg kg^{-1}; sample B is above the limit but the limit is within the MU; sample C is below the limit but the limit is within the MU; and sample D is below the limit and the limit is outside the uncertainty. Taking into account the acceptable level of probability of making a wrong decision (Williams, 2008), action will only be taken for sample A on the basis of the result and its MU, but not the result alone. MU can be reliably estimated using appropriate statistics if all possible sources of error component in the test method are known and identified.

The process of deriving MU and examples of the calculation are comprehensively discussed in the ISO GUM (ISO, 2008) and Eurachem Guides (Eurachem/CITAC, 2012). Basically, uncertainties of measurand occur in every procedure of the test method as well as from the associated references or standards that the analytical results are claimed traceable to. The ISO GUM 'bottom-up' estimation approach is based on the incorporation of all
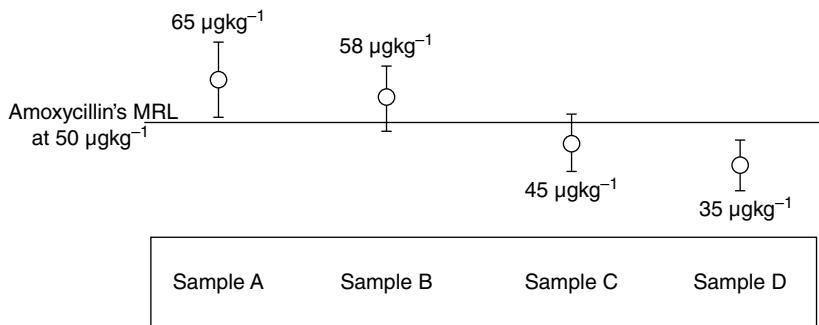
Figure 7.6   LC-MS/MS results of amoxycillin A in porcine muscle samples in four test samples. Taking into account the respective expanded uncertainty (error bar), only sample A is confirmed to be above the maximum residue limit (MRL).

potential sources of uncertainty (weighing, volumetric dilution of standards, calibration curves, drift of instrumental responses, etc.). Those uncertainties are eventually combined using the square root of the sum of the squares of individual error components to produce the combined standard MU (u_c) for the test method. MU is generally expressed as an expanded uncertainty which is obtained by multiplying u_c with a coverage factor k, and setting $k = 2$ at approximately the 95% confidence level. The Eurochem Guide also describes the 'top-down' approach which is based on the use of the performance parameters of method validation and collaborative studies. The two approaches have their merits and drawbacks. The bottom-up approach gives valuable insight into different sources of errors and offers potential improvements, but results may vary and depend on the estimations made. The top-down approach may result in an overestimated MU but it is easy to perform and allows method-specific uniform MU values. Nonetheless, laboratories should be able to work out a realistically good estimation in their analysis irrespective of which approach is used.

7.2.5 Laboratory accreditation

Laboratory accreditation is formal recognition by a third-party authority of a laboratory's capability to perform testing, measurement and calibration activities. The process benchmarks the building of a laboratory quality system and gives confidence to the laboratory's customers on the standards of service delivery. Another significant benefit is the international recognition of accredited test results from the mutual recognition arrangements among accreditation bodies. Although accreditation is a voluntary scheme, laboratories are recommended to seek accreditation for their fully validated analytical procedures (Tholen, 2011). In some countries and economies, in particular the EU, accreditation is a mandatory requirement for laboratories that are responsible for carrying out official testing of foods. For example, only accredited laboratories in the private sector in Hong Kong are eligible to apply for the outsourcing analytical service in foods from the local government.

7.3 Metrology in chemistry

MiC has developed from physical metrology and aims to achieve chemical measurements traceable to the SI or other well-defined reference standards having fully documented uncertainty estimation in accordance with the ISO GUM Guide (ISO, 2008). In coherence with ISO/IEC 17025, traceability, comparability and MU are the three fundamental pillars in MiC to ensure reliable chemical measurement, to promote fair trade and to support legislation (Kaarls, 2007). Historically, the concept of MiC was implemented at the signing of the Inter-Governmental Treaty of the Meter Convention in 1875. The development and infrastructure of metrology, including MiC, is briefly summarized in Table 7.4 and further information can be obtained at the BIPM official website.

NMI and DI maintain their national measurement standards for one or more quantities, and they are the key operational units within the MiC framework to support accurate and traceable measurements. Their major responsibilities include the development of measurement standards and measurement methods; maintenance of national calibration system and traceability hierarchy; dissemination of accurate measurement capabilities to field testing laboratories; and participation in inter-laboratory comparison programmes (Consultative Committee's key and supplementary comparisons) at the highest international level.

CCQM (Consultative Committee for Amount of Substance) programmes, in particular the key comparisons, are known to require demanding analytical skills and are exclusively participated in by NMI/DI or international organizations such as the International Atomic Energy Agency. Obtaining satisfactory results in the key comparison is used to benchmark the Calibration and Measurement Capabilities (CMC) in Appendix C under the International Committee for Weights and Measures Mutual Recognition Arrangement (CIPM MRA). Conferment of CMC to NMI/DI is based on the results in CCQM key comparisons together with the compliance of peer-review assessment and international quality systems.

Table 7.4   Infrastructure and development of metrology under the treaty of the Metre Convention

Structure	Functions
Metre Convention	Signed by 17 nations in 1875 to establish a permanent organizational structure for member governments to act in common accord on all matters relating to units of measurement. The treaty created a permanent scientific institute, the International Bureau of Weights and Measures (BIPM), which is under the exclusive supervision of the International Committee for Weights and Measures (CIPM). Representatives of the member states meet every fourth year at the General Conference on Weights and Measures (CGPM).
CGPM	Discusses and examines the work performed by BIPM. Makes appropriate recommendations on development and other major issues related to metrological measurements.
CIPM	Consists of 18 different Member States. Meets annually at the BIPM and undertakes preparatory work for technical decisions confirmed by the CGPM. It is supported by 10 consultative committees (CC) in which each CC is chaired by a representative of national metrology institute (NMI). The NMI for chemical measurement is the CC for Amount of Substance (CCQM). The CIPM Mutual Recognition Arrangement (CIPM MRA) was signed among national metrology NMIs and designated institutes (DI) in 1999. The Agreement relates to the establishment of the degree of equivalence of national measurement standards, and the mutual recognition of calibration and measurement certificates issued by participating institutes.
Regional Metrology Organizations (RMO)	Coordinates the metrological work of NMI within their region based on the specific regional needs and issues.
NMI and DI	Institutes established to develop and maintain measurement standards at the highest national level. Undertake primary realizations of the metrological base units and maintain a general overview of the national calibration and traceability hierarchy. Fundamental operation units to uphold the framework of the Metre Convention.

The CMC are the generally available internationally recognizable 'best' calibration and measurement capabilities of the testing institute. Accurate measurement systems can be disseminated through the traceability chain under the CIPM MRA to national field laboratories (Kaarls, 2004), commonly by means of CRM, technology transfer and the provision of proficiency testing (PT) programmes using assigned reference values for performance assessment (Figure 7.7).

Experience shows that accurate chemical measurement in foods is by no means easy. The removal of interferences arising from matrices, isolation of analytes from the matrix using often tedious sample pre-treatments before instrumental analysis, are the norm of food analysis. Inevitably, every step introduces errors and therefore both calibration of instruments and measurement procedure are deemed necessary.
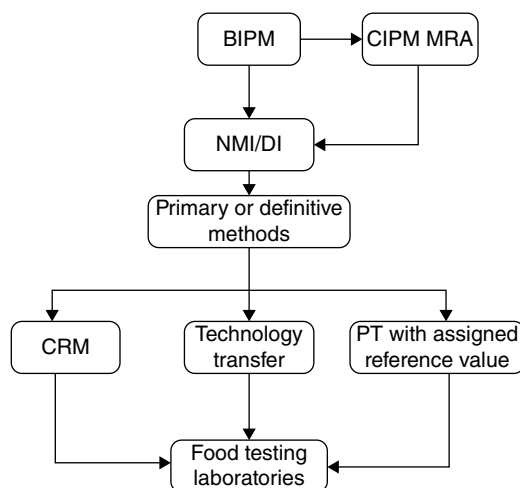
Figure 7.7   Provision of traceability and dissemination of MiC from NMI/DI to food testing laboratories through the CIPM MRA.

The calibrations involved in food analysis give rise to the development of CRM (standard and matrix) and proficiency testing programmes. The ISO/IEC 17025 and ISO Guide 33 (ISO, 2000) currently emphasize the unique usefulness of CRM in the calibration of instruments, validating analytical methods validation and traceability linkage. However, the metrological preparation of CRM and the assignment of property values and MU are sophisticated and resource-intensive processes in terms of knowledge, equipment and manpower as governed by the ISO Guides 34 (ISO, 2009) and 35 (ISO, 2006). Only a few institutions (relatively speaking) offer such high-calibre services (Ulberth, 2006; Wong *et al.* 2008).

As stated above, one of the core responsibilities of NMI and DI is using CRM as the national reference standards to disseminate accurate measurement to the field laboratories through their property values and the associated uncertainty statement. As a matter of fact, field laboratories have shown a higher preference for using CRM products manufactured by NMI/DI than those from commercial reference material producers. Firstly CRM from NMI and DI are well-characterized by primary methods or state-of-the-art measurement methods which provide to the user community the means of achieving chemical measurement quality assurance and traceability to national standards. Secondly, there is the prestigious recognition accorded to calibration and measurement certificates issued by these institutes under the CIPM MRA. Concerning the infinite diversity of measurands and matrices in the food sector, the quantity of CRM on the market is comparatively very limited. Demand always outstrips the supply such that NMI/DI need to cautiously prioritize suitable candidates in the CRM production list under limited resources on the basis of the national and regional requests. For instance, the release of CRM, SRM 3280 by NIST containing 13 vitamins, 24 elements and 2 carotenoids was to address the growing number of consumers on nutraceuticals and requests from the dietary supplement industry in the USA (Sander *et al.*, 2011). Since

Table 7.5 Major CRM producers from national metrology institutes or designated institutes

Country	NMI/DI
Australia	National Measurement Institute, Australia (NMIA)
Canada	National Research Council (NRC)
China	National Institute of Metrology (NIM)
European Union	Institute for Reference Materials and Measurements (IRMM)
Germany	Federal Institute for Materials Research and Testing (IRMM)
Japan	National Metrology Institute of Japan (NMIJ)
Korea	Korea Research Institute of Standards and Science (KRISS)
United Kingdom	Laboratory of the Government Chemist (LGC)
United States	National Institute for Standards and Technology (NIST)

laboratories benefit from validating their test methods with CRM, the production (hence the availability) of closely matched matrix CRM is likely to be an important task to deal with in the near future. Examples of some active NMI and DI (the major CRM producers) are provided in Table 7.5. Furthermore, a free-of-charge updated CRM list has been established on the internet (www.comar.bam.de), providing users with an easy method of checking the availability of the CRM they want to purchase.

Unknown sources of error present in the measurement process cannot be identified without relevant CRM, a frequent drawback in food analysis practice. PT is a useful means of detecting and initiating remediation when such problems arise, and it provides a useful platform by which participating laboratories can obtain an external and independent assessment of the accuracy of their results (Thompson *et al.*, 2006). In addition, improvement of existing and new methods and the subsequent resolution and reinforcement of customers' confidence in test results are among

the advatnages of continuous participation in PT. Accreditation bodies today require laboratories to use PT as a tool to demonstrate the credibility of their results from methods accredited in accordance with the requirements in the ISO/IEC 17025. From time to time, NMI/DI offer PT programmes to field laboratories primarily to support the national or international measurement systems and to improve their testing competence. The organization of the International Measurement Evaluation Programme (IMEP) by the Institute for Reference Materials and Measurements (IRMM) in EU (Lamberty *et al.*, 1996) is one of the many examples.

7.3.1   Assigned values in PT programmes

The assessment of participants' capability in PT programmes makes reference to the assigned values of the measurands in the test materials. The assigned values of PT programmes can be derived by a number of alternative approaches as listed in Annex B of ISO/IEC 17043 (ISO, 2010). These include, with increasing uncertainty: (1) known values, with results determined by specific proficiency test item formulation; (2) certified reference values, as determined by definitive test or measurement methods; (3) reference values, as determined by analysis, measurement or comparison of the proficiency test item alongside a reference material or standard, traceable to a national or international standard; (4) consensus values from expert participants (experts should have demonstrable competence in the determination of the measurand(s) under test); and (5) consensus values from participants. The operation and statistical analysis of PT programmes should meet the ISO/IEC 17043 requirements, and participants should be informed in advance concerning the choice and usage of the assigned values.

The majority of PT programmes, especially those operated by commercial scheme providers, are normally based on the consensus values. In general, the consensus values are less expensive to generate as they are directly obtained from participants' results using simple calculation and involve no further laboratory work. However, as the consensus mean originates from the pooled data, it might vary for each individual round when the same test materials are tested by different groups of laboratories. The quality of the consensus value as an assigned value is sometimes in question, simply because it might be influenced by some predictable factors such as the number of participants and their overall capabilities in the analysis. If this happens, the consensus value could result in bias from the 'true' value and lead to unreliable performance assessments.

Consider for example the Food Analysis Performance Assessment Scheme (FAPAS) PT programme 0754 on tin in a frozen tomato paste. The consensus value from 72 participating laboratories was 206 mg kg^{-1} and the reference value that was determined by a NMI using an IDMS primary method was 224.6 mg kg^{-1}. Due to the discrepancy of the two values, significant changes in participants' *z*-score results were reported in the programme. The results of 17 participants would need to be changed from satisfactory to unsatisfactory and 9 results from unsatisfactory to satisfactory if the consensus value is replaced by using the reference value (Kaarls, 2010). Erroneous assessment would occur if the consensus value deviates considerably from the true value, undermining the credibility of PT in quality assurance. PT providers should make judicious decisions on the assigned values such that meaningful interpretation of participant performance can be achieved.

A thorough comparison of the application of assigned values indicated that the quality of the assessment of the laboratories is better with a reference value (Baldan *et al.*, 2001). Concerning the important role of PT programmes in food analysis and the possibility of plausible bias in using consensus values, NMI/DI have strong preferences to use assigned reference values in their PT programmes. Two selected PT programmes with reference values on melamine in milk (Chu *et al.*, 2010) and cypermethrin in green tea (Dayarathna *et al.*, 2013) were chosen to explore the competence of food testing laboratories in the analysis of trace contaminants, as described in the following sections.

7.3.2   PT on melamine in milk

An international PT on melamine in milk organized in 2010 was registered with 76 laboratories from 28 countries; 68 of them returned results to the organizers. The assigned reference value for one of the test samples was 2.565 mg kg^{-1} which was the nominal mass fraction of melamine from gravimetrical spiking. The reference value was further confirmed by an LC-MS/MS method using an accurate IDMS technique (Hon *et al.*, 2011). Target standard deviation of the programme (at 0.315 mg kg^{-1} or 13.8%) was evaluated using the Horwitz equation and the *z*-score was used for the assessment of participants' performance. The distribution of participant data, ranging from 0.146 to 4.95 mg kg^{-1}, is shown in Figure 7.8. Consensus mean was 2.285 mg kg^{-1} with a negative deviation of 10.9% from the assigned reference value. Between-laboratory variation was 13.9% and 57 laboratories (85.1%) obtained satisfactory *z*-scores.

LC-MS/MS (75% of the laboratories) was found to be the most common technique for the quantification of melamine in the programme followed by GC-MS (13.2%), LC-UV (7.4%) and enzyme-linked immunosorbent assay (ELISA) (4.4%). As shown in Table 7.6, participants' performance correlated well with their used analytical techniques. Firstly, the mean result from LC-MS/MS users predominantly exhibited higher precision in terms of relative standard deviation and less bias from the reference values. Secondly, all LC-MS/MS and GC-MS users who employed isotopically labelled internal standards (e.g. $^{13}C_3{}^{15}N_3$-melamine, $^{13}C_3$-melamine or $^{15}N_3$-melamine) achieved *z*-score ≤ |2|. Furthermore, the usage of accredited methods had a positive influence on the reliability of results produced. There was only one unsatisfactory result out of the 20 laboratories who claimed to use accredited methods, but 3 questionable and 6 unsatisfactory results for the 42 non-accredited counterparts.
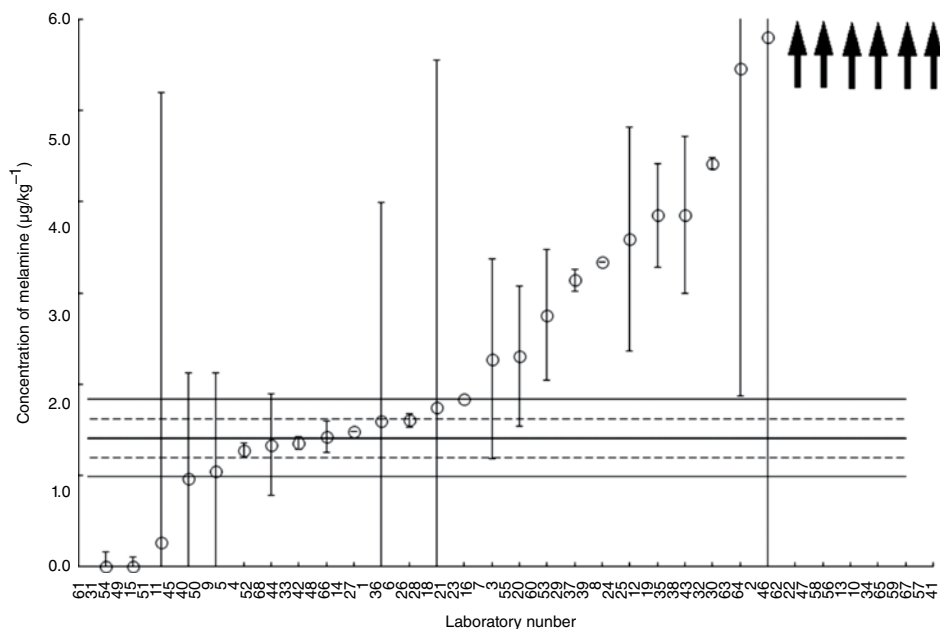
Figure 7.8 Distribution of participants' reported concentration (mg kg^{-1}) for melamine in milk. Central bold line is the reference assigned value, dotted lines represent |*z*|=1, solid lines represent |*z*|=2, error bars represent the respective expanded uncertainty and black circles indicate no MU reported.

Table 7.6 Participants' analytical methods and results in the PT programme for melamine in milk (––– no information reported; NA: not applicable; X_{ref}: assigned reference value; X_{robust}: mean calculated using robust test; CV: between-laboratory variation of participant results)

Lab. no	Instrument	Reported results (mg/kg)	MU (mg kg^{-1})	z–score	Accreditation status
1	GC-MS[3]	2.19	0.154	−1.1	No
2	LC-MS/MS[1]	2.507	0.352	−0.24	No
3	LC-MS/MS[3]	2.26	0.45	−0.93	No
4	LC-MS/MS[1]	1.984	0.381	−1.7	No
5	LC-MS/MS[1]	1.98		−1.7	Yes
6	LC-MS/MS[3]	2.23	0.669	−1.0	Yes
7	LC-MS/MS[3]	2.25	1.13	−1.0	Yes
8	LC-MS/MS[3]	2.37	0.24	−0.62	Yes
9	LC-MS/MS[3]	1.96	0.98	−1.8	Yes
10	LC-MS/MS[3]	2.71	0.542	0.33	No
11	LC-UV[1]	1.664	–––	−2.6	No
12	LC-MS/MS[3]	2.401	0.263	−0.53	No
13	LC-MS/MS[3]	2.684	0.064	0.26	No
14	LC-MS/MS[3]	2.179	0.604	−1.2	No
15	GC-MS[2]	1.194	0.001	−3.9	Yes
16	LC-MS/MS[1]	2.241	0.276	−1.0	No
17	GC-MS[1]	£2.0	0.06	NA	NA
18	LC-MS/MS[3]	2.22	0.33	−1.0	No
19	LC-MS/MS[3]	2.426	0.508	−0.46	Yes
20	LC-MS/MS[3]	2.27	0.18	−0.90	Yes
21	LC-MS/MS[3]	2.23	0.23	−1.0	–––
22	LC-MS/MS[2]	2.552	0.679	−0.11	No
23	UPLC-MS/MS[3]	2.23	0.1	−1.0	No
24	LC-MS/MS[3]	2.38	0.23	−0.59	No
25	LC-MS/MS[3]	2.40	0.46	−0.54	Yes
26	LC-MS/MS[1]	2.2	–––	−1.1	No
27	LC-MS/MS[1]	2.18	0.39	−1.2	No
28	LC-MS/MS[3]	2.2	0.1	−1.1	Yes
29	LC-MS/MS[3]	2.30	0.08	−0.82	No
30	LC-MS/MS[2]	2.5	0.09	−0.26	No
31	GC-MS[1]	0.280	0.042	−6.5	No
32	LC-MS/MS[3]	2.48	0.103	−0.31	No
33	LC-MS/MS[3]	2.11	0.22	−1.4	No
34	LC-MS/MS[3]	2.73	0.49	0.39	Yes
35	LC-MS/MS[3]	2.43	0.2	−0.45	Yes
36	LC-MS/MS[1]	2.199	0.429	−1.1	No
37	LC-MS/MS[3]	2.33	0.50	−0.73	No
38	LC-MS/MS[1]	2.46	0.93	−0.37	No
39	LC-UV[1]	2.34	0.1	−0.71	Yes
40	ELISA	1.91	–––	−1.9	–––
41	GC-MS[1]	4.95	–––	6.6	No
42	LC-MS/MS[2]	2.12	0.36	−1.3	No
43	LC-MS/MS[3]	2.46	0.74	−0.37	No
44	LC-MS/MS[1]	2.05	0.1	−1.5	–––
45	ELISA	1.898	0.135	−1.9	No

Lab. no	Instrument	Reported results (mg/kg)	MU (mg kg^{-1})	z-score	Accreditation status
46	LC-MS/MS[3]	2.54	0.2656	−0.15	Yes
47	GC-MS[3]	2.580	0.25	−0.03	No
48	LC-MS/MS[2]	2.15	0.13	−1.2	Yes
49	LC-MS/MS[1]	1.13	0.17	−4.1	No
50	GC-MS[1]	1.94	0.04	−1.8	Yes
51	LC-UV[1]	1.24	---	−3.8	No
52	LC-MS/MS[3]	2.018	0.02	−1.6	No
53	GC-MS[3]	2.287	0.115	−0.85	No
54	LC-MS/MS[1]	0.742	0.227	−5.2	No
55	LC-MS/MS[3]	2.26	0.601	−0.93	No
56	LC-MS/MS[2]	2.656	0.146	0.18	No
57	LC-MS/MS[1]	3.53	0.50	2.6	No
58	ELISA	2.626	---	0.10	Yes
59	LC-MS/MS[1]	2.964	0.128	1.0	Yes
60	LC-MS/MS[3]	2.28	0.11	−0.87	No
61	LC-MS/MS[1]	0.146	0.37	−6.9	No
62	LC-UV[1]	2.55	---	−0.12	No
63	LC-MS/MS[3]	2.5	0.2	−0.26	Yes
64	LC-MS/MS[1]	2.504	0.1202	−0.25	Yes
65	GC-MS[3]	2.75	0.39	0.44	No
66	LC-MS/MS[3]	2.178	0.112	−1.2	No
67	LC-MS/MS[1]	3.36	0.24	2.2	No
68	LC-UV[1]	2.03	---	−1.6	---
	X_{ref} (±MU)	2.565±0.354			
	X_{robust}	2.286			
	Median	2.260			
	CV (%)	13.9			

[1]Chromatographic methods without internal standard (IS);
[2]Employed non-isotopically labelled IS;
[3]Employed isotopically labelled IS.

Another technical issue requiring particular attention was the estimation of MU in which significantly large variations (ranging from 0.1% to 250%) irrespective of their used technique were reported. In addition, 10 laboratories had difficulty realizing the parameter and chose not to report.

7.3.3 PT on cypermethrin in green tea

The programme, organized by Regional Metrology Organization in the Asia Pacific in 2010, aimed to assess the actual capability of field laboratories providing analytical services for incurred pesticide residues in tea in the region. One of the four measurands was cypermethrin. Target standard deviation was set at 15% and the assigned reference value (141 ng g^{-1}) was derived from the weighted mean of the results determined by three NMI using GC-IDMS techniques. Both z-scores and E_n-scores were used as the performance indicators in the programme. A total of 42 laboratories registered for the programme and 31 laboratories from 12 countries returned results to the organizers. Since most of the laboratories were from the developing countries, a training workshop on quality assurance was provided to all participants in order to reinforce their understanding on

method validation and MU estimation before the commencement of the programme.

The reported results are illustrated in Figure 7.9 and summarized in Table 7.7. The majority of the participants used GC-ECD for the measurement and the remaining used the more selective GC-MS and GC-HRMS instruments. However, the variation of individual data was extraordinarily large by 7 orders of magnitude. The robust mean (X_{robust}) was 231 ng g^{-1} which was 64% higher than the reference value, and the between-laboratory variation was up to 85%. In fact, such variability has previously been encountered in other PT programmes on the analysis of pesticide in plant species (Şenyuva and Gilbert, 2006; Kong $et\ al.$, 2007). The extracts of tea were known to contain a large quantity of endogenous substances and pigments that could potentially mask the target pesticides, and extensive clean-up was needed. Furthermore, the analysis of trace concentrations of cypermethrin is definitely a challenge because of the presence of four chromatographically resolved pairs

of isomers (Sin $et\ al.$, 2012) in the compound. If a good chromatographic separation of the cypermethrin isomers and an adequate removal of matrix interference were not obtained, the analyst could end up with dubious measurements.

The programme clearly demonstrated these challenges as only 7 out of the 31 laboratories achieved satisfactory z- and E_n-scores. Again, all the participants who used isotopically labelled internal standards in their methods obtained satisfactory results and outperformed other participants. It is also noteworthy that unreasonable results can be avoided if participants exercised their analytical common sense. For example, cypermethrin and other pesticides at the 0.02 ng g^{-1} level (Lab. No. 569) could barely be detected by GC-ECD or GC-MS. Difficulty in presenting good MU estimation (ranging from 0.1% to 3259%) was also identified in this programme, although participants reported that the uncertainties of analysis were consistently due to recovery and precision of spiked samples.
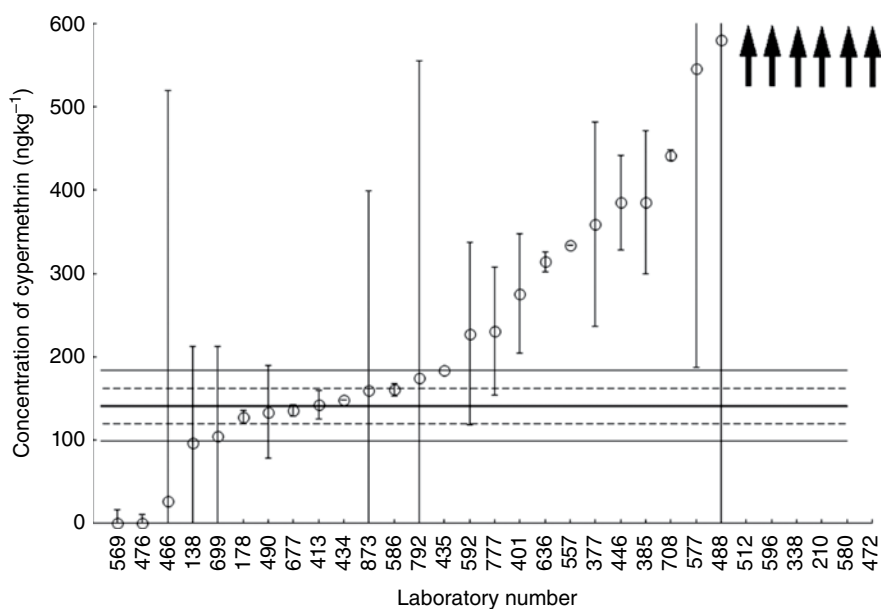
Figure 7.9   Distribution of participants' reported concentration (ng g^{-1}) for cypermethrin in green tea. Central bold line is the reference assigned value, dotted lines represent $|z| = 1$, solid lines represent $|z| = 2$, arrows show the reported results are off scale and error bars represent the respective expanded uncertainty.

Table 7.7 Participants' analytical methods and results in the PT programme for cypermethrin in green tea (–––: no information reported; SPE: solid phase extraction; GPC: gel permeation chromatography; NA: not applicable; X_{ref}: assigned reference value; X_{robust}: mean calculated using robust test; CV: between-laboratory variation)

Lab. no	Instrument	Clean-up	Reported results (ng g⁻¹)	MU (ng g⁻¹)	z-score	E_n-score
138	GC-MS	SPE	97	16.3	−2.1	−1.9
178	GC-MS[1]	Florisil	127.4	10.0	−0.64	−0.7
210	GC-ECD	SPE	1742.09	491.85	76	3.3
338	GC-MS	Silica gel	1020.4	116	42	7.5
377	GC-ECD	Florisil	359.01	107.58	10	2.0
385	GC-ECD	Florisil	386	8.03	12	13.7
401	GC-ECD	SPE	276	56	6.4	2.3
413	GC-HRMS[1]	GPC, SPE	142.5	7	0.07	0.1
434	GC-HRMS[1]	GPC, SPE	148	17	0.33	0.3
435	GC-MS	SPE	183.86	0.129	2.0	2.7
446	GC-ECD	SPE	385	240	12	1.0
466	GC-ECD	–––	27.14	7.49	−5.4	−6.4
472	GC-ECD	–––	10927.39	381.43	510	28.3
476	–––	–––	0.43	0.009	−6.6	−8.8
488	GC-ECD	Florisil	580	109	21	4.0
490	GC-ECD	SPE	134	77	−0.33	−0.1
512	–––	–––	680	72	25	7.3
557	GC-ECD	Florisil	334.3	12.07	9.1	9.6
569	GC-ECD	Silica gel	0.02	0.002	−6.7	−8.8
577	GC-ECD	Silica gel	546.43	122.40	19	3.3
580	GC-ECD	Florisil	3161.63	56.68	143	51.3
586	GC-ECD	yes	160.6	85.98	0.93	0.2
592	GC-ECD	Florisil	227.86	6.250	4.1	5.1
596	GC-ECD	GPC	921.14	359.24	37	2.2
636	GC-ECD	SPE	314	3144	8.2	0.1
678	GC-MS[1]	SPE	135.7	9.5	−0.25	−0.3
699	GC-MS	GPC	104.9	10.7	−1.7	−1.9
704	GC-MS	Florisil	–––	–––	NA	NA
708	GC-ECD	–––	441.9	7.1572	14	17.2
777	GC-ECD	GPC	230.75	24.17	4.2	3.1
792	–––	–––	174.2	89.1	1.6	0.4
873	GC-ECD	yes	159.6171	2.3308	0.88	1.2
		X_{ref} (±MU)	141±16			
		X_{robust}	323			
		Median	231			
		CV (%)	85.4			

[1]Employed isotopically labelled internal standard

7.3.4   Insights from the two described PT

The two PT programmes described above offered the advantage of using assigned reference values with minimal bias from the true values for performance assessment of participating laboratories. Unlike consensus values, which only compare the results within the population in the programme and theoretically result in 95% of participants achieving satisfactory z-score results, the assessments using the known value for melamine and weighted mean value from expert NMI/DI for cypermethrin were made on the basis of metrological approach but not the application of statistics. The evaluation of the performance of field laboratories relied on the direct comparison of their reported values with the reference value, and the results reflect the actual competence of the participants in analysing the measurands in the test materials.

Results of the two PT examples showed the presence of large analytical variability in food testing from field laboratories. The variability is observed to be dependent on the complexity of matrices, the concentration and nature of analytes and the analytical methods. Low concentrations of incurred pesticides such as cypermethrin and the potential co-extracted interferences in tea increased the difficulty in obtaining reliable analysis for most of the field laboratories. A significant number of pesticide results showed a poor comparability, possibly due to the fact that the widely accepted quality control and metrological measurement systems are not fully implemented in those laboratories. Also, in many cases, the uncertainty of the whole analytical procedure was not comprehensively understood.

These findings send out a clear message that more effort has to be deployed by relevant food and analytical authorities to improve the situation. On the other hand, laboratories are encouraged to participate in PT with reference assigned values whenever they would like to improve their measurement and check their actual testing capability.

7.4   Conclusions and future outlook

Good decision-making depends on the availability of good-quality data. To achieve a reliable measurement system in food analysis, it is necessary that laboratories comply with the internationally agreed harmonized quality requirements (e.g. ISO/IEC 17025) such that the establishment of traceability and comparability in their testing procedures is ensured. All analytical methods should be validated for fitness for purposes and the use of control charts for on-going process monitoring is required whenever necessary. The associated measurement uncertainties should be clearly defined and properly estimated. Traceable calibration standards such as CRM (including standard and matrix CRM) should always be used to ensure an unbroken chain of traceability in the procedure. PT is an invaluable external tool for quality assurance; continuous participation in PT, in particular those using reference assigned values, can therefore offer unique advantages to laboratories to identify method deficiency, improve testing competency and the prerequisites for laboratory accreditation. The implementation of MiC concept also supports accurate measurement and enhances global recognition of analytical results. Food laboratories should seek technical assistance from their NMI/DI through appropriate dissemination of measurement traceability mechanisms within the MiC framework.

Acknowledgements

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8

Protection of the Agri-Food Chain by Chemical Analysis: The European Context

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Summary

The present chapter describes the objectives and basis of European food and feed law. We discuss how new food and feed law is developed in the EU and how it works in day-to-day regulation in the United Kingdom both with regard to domestic production and imported food. This is illustrated with EU legislation and typical analysis for: (1) contaminants, for example, the genotoxic carcinogenic fungal metabolites, aflatoxins and other mycotoxins; (2) veterinary residues, for example, carcinogenic nitrofurans; and (3) aluminium in imported noodles. We describe the context (potential impact on consumers), the law and the nature of the chemical analysis undertaken to protect consumers and responsible traders. Finally, we focus on situations where disagreements arise between laboratories acting for the regulator and for the trader and how these are resolved in the UK.

8.1 Introduction

Agri-food is a global business. The European Union (EU) is currently the world's largest trader in food and drink with imports of \$74.8 billion (15.9% world share) and exports of \$87.2 billion (17.8% world share) in 2010, although its export figures, along with almost all developed countries, are in slow decline in favour of emerging economies (FoodDrink Europe 2011a). The European Union is based on a set of laws and a standard decision-making procedure known as the 'ordinary legislative procedure' that gives the same weight to the European Parliament and the Council of

the European Union on a wide range of areas including trade, food, animal feed, the environment and consumer protection.

The European Commission is the civil service of the EU and consists of the 'Commissioners' selected by the Member States and, below them, the Directorates General (DGs). DG SANCO, the Directorate General for Health and Consumers, takes primary responsibility for food safety issues. The European Commission represents the interests of the EU as a whole. It proposes and drafts new legislation to the European Parliament and the Council of the European Union, and it ensures that EU law is correctly applied by member countries. The vast majority of European laws are adopted jointly by the European Parliament and the Council. This means that the directly elected European Parliament has to approve EU legislation together with the Council, representing the governments of each of the EU countries (European Union, 2013a). The Court of Justice of the European Union interprets EU law so that it is applied in the same way in all EU countries. It also settles legal disputes between EU governments and EU institutions. Individuals, companies or organizations can also bring cases before the Court if they feel their rights have been infringed by an EU institution (European Union, 2013b).

There are five types of legal measures in the EU: Regulations, Directives, Decisions, Recommendations and Opinions. EU legal measures can be accessed at Eur-Lex, Access to European Union law (http://eur-lex.europa.eu/en/index.htm).

A 'Regulation' is a binding legislative act. It must be applied in its entirety across the EU. A 'Directive' is a legislative act that sets out a goal that all EU countries must achieve. However, it is up to the individual countries to decide how. Much food law that was in the past governed by Directives is increasingly subject to Regulations, for example food labelling formerly under Directive 2000/13/EC of 20 March 2000 is now controlled by Regulation (EU) No. 1169/2011 of 25 October 2011 (EU, 2011a).

A 'Decision' is usually quite specific and is directly binding on those to whom it is addressed

(e.g. any or all of the EU countries or an individual company). For example, Commission Implementing Decision 2013/287/EU of 13 June 2013 amending Implementing Decision 2011/884/EU on emergency measures regarding unauthorized genetically modified rice in rice products originating from China (EC, 2013a). Decision 2011/884/EU requires all rice consignments imported into the EU from China to be tested for the presence of molecular markers and elements often associated with genetic modification. Decision 2013/287/EU amends some aspects of 2011/884/EU as regards prior notification to EU authorities of consignments in transit and sampling provisions (Burns *et al.* 2013; EC, 2013a).

A 'Recommendation' is not binding; it allows the European institutions to make their views known and to suggest a line of action without imposing any legal obligation on those to whom it is addressed. An example is Commission Recommendation 2012/154/EU of 15 March 2012 (EC, 2012) on the monitoring of the presence of ergot alkaloids in feed and food. Ergot is a fungal structure (*Claviceps* species) that paracitises grain or grasses and the ergot alkaloids are among the most important natural pharmaceuticals and toxins in human history. Unknowing ingestion has led to mass poisoning with dire physiological and social implications (Schardl *et al.*, 2006). More data were needed on the variability in ergot alkaloid patterns in individual plant species and the EU Commission recommended that Member States should, with the active involvement of feed and food business operators, monitor the presence of ergot alkaloids in cereals and cereal products intended for human consumption or intended for animal feeding (EC, 2012).

An 'Opinion' is not binding. It can be issued by the main EU institutions (Commission, Council, Parliament), the Committee of the Regions and the European Economic and Social Committee. While laws are being made, the committees give opinions from their specific regional or economic and social viewpoint. An example is the Opinion of the European Economic and Social Committee on the 'Proposal for a regulation of the European

Parliament and of the Council on food intended for infants and young children and on food for special medical purposes' (EU, 2011b).

Due to food crises such as BSE (Bovine spongiform encephalitis, so-called 'mad cow disease') and dioxins in food, there has been a high salience of food safety in Europe for many years with corresponding prominence on the political agenda. As a consequence a new approach, 'from farm to table', was proposed in the EU white paper on food safety (EC, 1999). The new approach brought about the foundation of the European Food Safety Authority (EFSA) and overarching laws on food safety, quality and traceability (Grunert, 2005). That these are not just European issues is self evident but in emphasis, following the World Health Organization Forum in November 2007, the Beijing Declaration recognized the importance of food safety along with the rights of all individuals to a safe and adequate diet.

The European context in which food safety is regulated can be considered to impact on non-EU countries in two main ways: (a) an understanding of EU law and practice is essential in order to export food to the EU; and (b) consumers in emerging economies are becoming more demanding and critical about their food choices. Policy makers can look to the means by which the EU has addressed similar issues and perhaps gain from an appreciation of the strengths and weaknesses of the EU approach.

This chapter looks at the main elements of EU food and feed law and discusses key areas of scientific practice in the area of *chemical* food safety.

8.2   European food and feed law

The twin objectives of the European Union's food safety law are (a) to protect consumers' health and interests and (b) to guarantee the smooth operation of trade in the single European market. Consumers' interests include food quality and choice as well as safety and also encompass animal welfare; labelling therefore plays an important part in EU food law. Specific issues of food hygiene (microbiology), animal health and welfare and plant health are not dealt with in this chapter, which instead concentrates on the chemical aspects of food safety and standards.

Food and animal feed must be safe, authentic and properly labelled, responsibility for which falls to those who make and sell it. There is, however, a public expectation of regulatory oversight by government. Regulation of food and feed has a long history but as the 20th century ended a series of food scandals in Europe, particularly BSE (Phillips, 2000) and a highly significant dioxin contamination in Belgium in 1999 (Covaci, 2008), resulted in intensified efforts to restore confidence in food safety and a sea-change in European food control law. The new approach, 'From the Farm to the Fork', is intended to guarantee a high level of safety for foodstuffs and food products marketed within the EU, at all stages of production and distribution, and involves both food products produced within the EU and those imported from non-EU countries (EU, 2013c).

Two key pieces of legislation were enacted that continue to influence not only Europe but countries that export to it. Regulation 178/2002 laid down the general principles and requirements of food law, established the European Food Safety Authority (EFSA) and set up new procedures in matters of food safety. Article 14 of 178/2002 makes clear the food safety requirements: (1) food shall not be placed on the market if it is unsafe; and (2) food shall be deemed to be unsafe if it is considered to be either injurious to health or unfit for human consumption. Regulation 178/2002 was followed and supplemented by Regulation 882/2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. This Regulation is designed to augment existing legislation on official control of food and feed by a harmonized Community approach to the design and implementation of national control systems. The purpose of Regulation 882/2004 is: to prevent or eliminate

risks which may arise (either directly or via the environment) for human beings and animals, or reduce these risks to an acceptable level; and to guarantee fair practices as regards trade in food and feed and the protection of consumers' interests, including labelling of food and feed. Regulation (EC) No 882/2004 has been in application since 1 January 2006. Feedback has shown that adjustments are necessary to simplify and clarify the legal framework related to official controls, and consolidate the integrated approach in all areas related to the food chain. At the time of writing Regulation 882/2004 is under review. In particular, it is proposed to alter the financing of official controls, consolidate official controls on residues of veterinary medicines, on import of live animals and products of animal origin and modernize animal health and plant health law and the rules on plant reproductive material (UK FSA, 2013a).

A key tool in promoting food safety in the EU is the Rapid Alert System for Food and Feed (RASFF). RASFF was put in place to provide food and feed control authorities with an effective way to exchange information about measures taken in response to serious risks detected in relation to food or feed. This exchange of information helps Member States to act more rapidly and in a coordinated manner in response to a health threat caused by food or feed. In 2012 almost 50% of RASFF notifications were related to food and feed rejections at EU borders (RASFF, 2013).

In the following sections we highlight significant areas of food and feed safety in which chemical analysis plays a major role. Some of the topics considered in this chapter, such as the chemical contaminants aflatoxins and aluminium, feature heavily in RASFF; consequently, they are considered to be either a known or an emerging 'high risk' to public health. Hence, pursuant to Regulation 669/2009 (implementing Regulation (EC) No. 882/2004 of the European Parliament and of the Council as regards the increased level of official controls on imports of certain feed and food of non-animal origin and amending Decision 2006/504/EC), certain feed and food of non-animal origin from certain non-EU countries can only enter the EU through specific ports and airports approved as designated points of entry (DPEs) where enhanced official controls (including sampling and analysis) will be carried out. For example, Regulation 669/2009 currently requires 10% of consignments of dried noodles imported into the EU from China to be sampled and analysed for aluminium (UK FSA, 2013b). Other topics such as the veterinary residues of nitrofuran metabolites are known to give problems for countries exporting to the EU.

8.3   Chemical contaminants

Chemical contaminants are substances not intentionally added to food and may arise as a result of production, packing, transport, storage, cooking or as a result of environmental contamination. In order to prevent risks to human health, the EU has taken measures to regulate the concentrations of certain contaminants in food.

Regulation (EEC) No. 315/93 prohibits the marketing of foodstuffs containing an unacceptable amount of residual contaminants, present in food as a result of treatment after production or through environmental contamination and likely to pose a hazard to public health. Regulation 315/93 does not apply to contaminants which are the subject of more specific rules (see Table 8.1) or to extraneous matter such as insect fragments, animal hair, etc. EU Member States (MS) may not prohibit trade in foods which comply with Regulation 315/93, but may take restrictive measures if a contaminant poses a hazard to public health. In these circumstances MS must inform the other MS and the Commission immediately, giving reasons for any decision. The Commission will consult the Standing Committee on the Food Chain and Animal Health (SCoFCAH) which assists the Commission on all matters which concern contaminants, including the establishment of authorized maximum tolerances. Standing Committees are made up of representatives from EU governments and public authorities

Table 8.1 Contaminants regulated in the EU

Type	Contaminant
Mycotoxins	Aflatoxins B_1 and the sum of B_2, G_1 and G_2; aflatoxin M_1 in milk; ochratoxin A; patulin; deoxynivalenol; zearalenone; fumonisins; T-2 and HT-2 toxin
Heavy metals	Lead; cadmium; mercury; tin (inorganic);
Agricultural inorganic residues	Nitrates
Chloropropanols	3-Monochloropropane-1,2-diol (3-MCPD)
Complex and aromatic organics	Dioxins and polychlorinated biphenyls (PCBs); polycyclic aromatic hydrocarbons (PAHs)

Table 8.2 Patulin maximum limit (maximum permitted concentration, Regulation 1881/2006; EC, 2006a)

Commodity	Patulin: maximum (μg kg^{-1})
Fruit juices, fruit nectars, spirit drinks and other fermented drinks from apples	50
Solid apple products, including apple compote, apple puree intended for direct consumption with the exception of foodstuffs listed below	25
Apple juice and solid apple products, including apple compote and apple puree, for infants and young children and labelled and sold as such	10.0
Baby foods other than processed cereal-based foods for infants and young children	10.0

and play a key role in ensuring that EU decisions and regulations on food and feed safety, animal health and welfare and plant health are practicable and effective. SCoFCAH covers the entire food supply chain, from animal health issues on the farm to the product on the consumer's table and is chaired by a European Commission representative (SCoFCAH, 2013).

Specific contaminants (Table 8.1) are regulated by Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels in foodstuffs (EC, 2006a). There are other contaminants, for which no regulations as yet exist, which are the subject of other measures (see Table 8.5 page 138). We will consider the mycotoxin contaminants in detail.

For further information on the above and other contaminants see the website of the European Commission (http://ec.europa.eu/food/food/chemicalsafety/contaminants/index_en.htm).

8.3.1 Mycotoxins

Mycotoxins are relatively low-molecular-weight chemical compounds formed as secondary metabolites by filamentous fungal species that readily colonize crops and contaminate them with toxins in the field or after harvest. Bhat *et al.* (2010) and Zaki *et al.* (2012) have reviewed the potential hazards of several mycotoxins – aflatoxins, zearalenone, ochratoxins, and fumonisins – commenting on their specific fungal origin, economic significance and prevention strategies for fungal and mycotoxin contamination.

8.3.1.1 Aflatoxins

Aflatoxins are secondary metabolites of moulds such as *Aspergillus flavus* (from which their name derives) and *Aspergillus parasiticus* and are genotoxic carcinogens (EC, 1996) capable of inducing liver cancer, particularly

with simultaneous hepatitis B virus infection. They are among the most potent mutagens known (Herrman and Walker, 1999). Genotoxic carcinogens pose particular problems in regulating for food safety and Barlow *et al.* (2006) give a readable account of the risk assessment of substances that are both genotoxic and carcinogenic. Aflatoxins were originally identified in the early 1960s arising from a major outbreak in England of 'Turkey X disease' and subsequent toxic episodes in species other than poultry (Clifford and Rees, 1967). Although there are numerous analogues, four naturally occurring aflatoxins are recognized in legislation (B_1, B_2, G_1 and G_2), denoting their retention factor R_f and blue or green fluorescence under UV light on thin layer chromatography (TLC) plates which was the original means of their detection. In addition, aflatoxin M_1 occurs in milk arising from metabolism of other aflatoxins in the animal's feed. Aflatoxins contaminate a variety of staple foods, particularly maize and groundnuts but also spices, figs and many other crops. Aflatoxin B_1 is the most toxic and usually the most abundant of the natural analogues. Human mortality and morbidity from aflatoxicosis are well known, for example in Kenya in 2004 125 deaths occurred due to aflatoxins in maize. Despite this, the adverse public health effects of continuous and often high-level mycotoxin exposure in many low-income countries receives little attention (Wild and Gong, 2010).

Regulation 1881/2006 sets out maximum concentrations (limits) for aflatoxin B_1 and total aflatoxins (the sum of B_1, B_2, G_1 and G_2) in specified food commodities. For example there are maxima of 2.0 µg kg^{-1} for aflatoxin B_1 and 4.0 µg kg^{-1} for total aflatoxins in groundnuts (peanuts) and other oilseeds for direct human consumption or use as an ingredient in foodstuffs. Groundnuts and other oilseeds that are to be subjected to sorting or other physical treatment before human consumption or use as an ingredient in foodstuffs (with the exception of groundnuts and oilseeds for crushing for refined vegetable oil production) may not contain more than 8.0 µg kg^{-1} aflatoxin B_1

and 15.0 µg kg^{-1} total aflatoxins. The latest version of the regulation (EC, 2006a) should be consulted for up-to-date limits. An interesting situation arose in 2008, when the *Codex Alimentarius* set a maximum level of 10 µg kg^{-1} for total aflatoxins in ready-to-eat almonds, hazelnuts and pistachios, a concentration higher than that then in force in the EU (4 µg kg^{-1} total aflatoxins). After careful consideration, including an expert risk assessment by EFSA (EFSA, 2006, 2010), the Commission concluded that public health would not be adversely affected by increasing the levels for total aflatoxins from 4 µg kg^{-1} to 10 µg kg^{-1} for almonds, hazelnuts and pistachios. However, a limit of 8 µg kg^{-1} was retained for aflatoxin B_1 in both almonds and pistachios with a limit of 5 µg kg^{-1} for aflatoxin B_1 in hazelnuts (all for direct human consumption or use as an ingredient in foodstuffs), thus bringing EU law into effective line with Codex. EFSA and the Commission reiterated the importance of reducing the number of highly contaminated foods reaching the market. For further information see the European Commission website at http://ec.europa.eu/food/food/chemicalsafety/contaminants/aflatoxins_en.htm.

Methods of sampling and performance criteria for analysis for the official control of mycotoxins, including aflatoxins, are laid down in Commission Regulation No. 401/2006 (EC, 2006b). This ensures that the same sampling criteria intended for the control of mycotoxin content in food are applied to the same products by the competent authorities throughout the EU and that certain analytical performance criteria, such as recovery and precision, are fulfilled.

Since fungal contamination is patchy, aflatoxins are known to be inhomogenously distributed in consignments necessitating incremental sampling at import, followed by high shear mixing with a defined amount of water to form a slurry. For example, the method laid down in Regulation No. 401/2006 (EC, 2006b) for groundnuts (peanuts), other oilseeds, apricot kernels and tree nuts for analysis for aflatoxins requires an incremental sample of about 200 g (typically 100 g for other commodities). Depending on the size of the consignment (≤0.1 tonne to >15 tonnes), 10–100

incremental samples are taken. Consignments of over 500 tonnes must he assigned into 100 tonne sub-lots. The regulations must be consulted for detail but it can be seen that for typical consignments of 15 tonnes and more, an aggregate sample of 20 kg is required.

The importance of forming a water slurry to achieve aflatoxin homogeneity and aid in subsequent analysis was recognized 37 years ago (Velasco and Morris, 1976) and has been affirmed over the years (Whitaker *et al.*, 1980; CEN 2006; Spanjer *et al.*, 2006), but may still be poorly understood by some exporters seeking to control aflatoxin concentrations. Following incremental sampling and slurrying, the sample is divided into multi-part portions. Each of the multi-part portions are again divided to form portions for the enforcement, trade and, if required, the reference (referee; see Section 8.4) analysis. If one of the multi-part portions fails to meet statutory limits the consignment is rejected.

Analysis for mycotoxins typically begins with extraction of the sample, for example by liquid–liquid extraction, supercritical fluid extraction or solid phase extraction followed by immunoaffinity column clean-up and separation methods such as liquid chromatography, gas chromatography or capillary electrophoresis. Rapid methods such as enzyme linked immunosorbent assay (ELISA) are available (Turner *et al.*, 2009). Aflatoxins are typically extracted with acetonitrile/water or methanol/water depending on the matrix, followed by immunoaffinity column clean-up and liquid chromatography with post-column derivitization (bromination or Kobra cell) and fluorescence detection (Papadopoulou-Bouraoui *et al.*, 2002). Sample extracts, together with solvent standards, pre-extraction and post-extraction matrix spikes, may also be analysed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), which also confirms the molecular identity and presence of aflatoxin B_1 and other analogues if present (Theodosis and Colwell, 2007; Theodosis *et al.*, 2008). Analytical quality control is essential in performing analysis if valid results are to be obtained. Guidance is available, for example on quality control (Thompson and Wood, 1995) and single laboratory validation of methods of analysis (Thompson, Ellison and Wood, 2002).

When analytical results are obtained they should be recovery corrected and statistically assessed to derive their measurement uncertainty (MU). Further details are given elsewhere in this book (*Achieving Quality Chemical Measurements in Foods*, Chapter 7). While practices differ even within the EU, it is considered best practice by the authors when interpreting a result against a legal standard to use the mean result minus its measurement uncertainty, calculated as a 95% confidence interval. Rounding of results must also be considered. Further advice on these issues is freely available from the European Commission and the Joint Committee for Guides in Metrology (EC, 2004, 2010; BIPM 2013).

8.3.1.2 Ochratoxin A

The ochratoxins are also metabolites of fungal genera such as *Aspergillus* and *Penicillium*. Ochratoxin A (OTA) was discovered in 1965 by South African scientists as a toxic secondary metabolite of *Aspergillus ochraceus*. Of the ochratoxins, OTA is the major metabolite of toxicological significance, mainly as a contaminant of cereal grains (corn, barely, wheat and oats). It has also been found in beans (soyabeans, coffee, cocoa), peanuts and meat in some countries. Ochratoxin A gives rise to characteristic renal pathologies (EFSA, 2006, 2010), is teratogenic in rat, hamster and chick embryo and is an inhibitor of hepatic mitochondrial transport causing damage to the liver, gut, lymphoid tissue and renal tubular damage (Zaki *et al*. 2012). Regulation 1881/2006 (EC, 2006a) lists permitted maxima of $2.0–10.0\ \mu g\ kg^{-1}$ for wine, cereals, cereal products, coffee and other commodities. A reduced limit of $0.5\ \mu g\ kg^{-1}$ applies for food for infants and young children while higher limits ($15\ \mu g\ kg^{-1}$) apply to spices, except for *Capsicum* species where a proposed reduction of the limit for ochratoxin A in *Capsicum* spp. (dried fruits thereof, whole or ground, including chillies, chilli powder, cayenne and paprika) from $30\ \mu g\ kg^{-1}$ to $15\ \mu g\ kg^{-1}$ has been postponed until January 2015 (see Regulation 1881/2006 for further details;

EC, 2006a). Analysis by extraction, immunoaffinity column clean-up followed by liquid chromatography with fluorescence detection and by LC-MS/MS is described by Molinie *et al*. (2005) and Theodosis and Colwell (2007).

8.3.1.3   Patulin

The mycotoxin patulin is a small (154 Da) lactone-containing secondary metabolite produced by fungi belonging to several genera, including *Penicillium*, *Aspergillus* and *Byssochlamys* species. It is soluble in water, ethanol, acetone, ethyl acetate, ethyl ether and chloroform, and it is stable to heat processing at pH < 6. Patulin is gradually destroyed during storage in the presence of sulphites and ascorbic acid and is completely degraded in 15 seconds in aqueous solution by

10% ozone. Fermentation of apple juice to produce alcoholic beverages is said to destroy patulin and there is a maximum permitted concentration in such products. Although patulin can occur in many mouldy fruits, grains and other foods, the major sources of patulin contamination are apples and apple products, particularly those made from poor-quality fruits (damaged and rotting fruits). A limit of 50 μg kg^{-1} is widespread in many jurisdictions (see Tables 8.2 and 8.3).

The UK Committee on Mutagenicity has classified patulin as mutagenic. A review by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that it has no reproductive or teratogenic effects, but shows embryotoxicity accompanied by maternal toxicity. Based on available experimental results, it was concluded that patulin is

Table 8.3   Non-exhaustive summary of typical EU maximum limits (ML) for mycotoxins in foods. Three examples are given: the commodity with the lowest ML; a typical staple food ML; and the commodity with the highest ML. The original regulations must be consulted for all maxima in force (http://eur-lex.europa.eu/Notice.do?val=437851:cs&lang=en&list=572915:cs,437851:cs,&pos=2&page=1&nbl=2&pgs=10&hwords=) for the latest current consolidated version (dated 12.03.2012) from which the following data were obtained.

	Typical EU Maximum Limits (ML). Units are micrograms per kilogram, μg kg^{-1} for commodity for direct human consumption or use as an ingredient in foodstuffs					
	Commodity with lowest ML	ML	Typical staple foods	ML	Commodity with the highest ML	ML
Aflatoxin B$_1$	Foods for infants and young children	0.10	Certain cereals, cereal products, dried fruit, peanuts, tree nuts	2.0	Almonds, pistachios and apricot kernels	8.0
Sum of aflatoxins B$_1$, B$_2$, G$_1$, G$_2$		-		4.0		10.0
Aflatoxin M$_1$		0.025		-		-
Ochratoxin A	Foods for infants and young children	0.50	Certain cereal products	3.0	Liquorice extract	80
Patulin	Foods for infants and young children	10.0	Apple compote, or puree	25	Fruit juices	50
Deoxynivalenol	Foods for infants and young children	200	Bread	500	Unprocessed maize, durum wheat and oats	1750
Zearalenone	Foods for infants and young children	20	Bread	50	Refined maize oil	400
Fumonisins	Foods for infants and young children	200	Maize breakfast cereals & snack	800	Unprocessed maize	4000
T-2 and HT-2 toxin (Note: recommendation only as yet)	Cereal foods for infants and young children	15	Breakfast cereals	75	Oats (with husk)	1000

genotoxic, but that no adequate evidence existed for carcinogenicity in experimental animals. A recent review gives further details (Puel *et al.*, 2010).

Analysis for patulin usually involves extraction into ethyl acetate followed by clean-up and liquid chromatography (EC, 2002, which also gives dietary intakes and other data). Sargenti and Almeida 2010 have briefly reviewed methods for patulin and recommended a rapid method combining sonication and ethyl acetate liquid extraction followed by liquid chromatography with UV detection; LC-MS confirmation is also possible.

8.3.1.4 Deoxynivalenol

The remaining EU regulated mycotoxins, deoxynivalenol (DON), fumonisins, T-2 and HT-2 toxin and zearalenone are mainly produced by moulds of the *Fusarium* group (but also by other fungal genera, including *Trichoderma*, *Stachybotrys*, *Verticimonosporium*, *Cephalosporium* and *Myrothecium*). Deoxynivalenol (DON) is the most widespread of the 'trichothecenes', sesquiterpene epoxides with a tricyclic 'trichothecene' nucleus. The epoxide moiety appears essential for toxicity. First isolated from the mould *Trichothecium roseum* in 1948 (Desjardins *et al.*, 1993), there are approximately 180 known naturally occurring trichothecenes (EFSA, 2011) with varying chemical structures but only a limited number have been shown to be agriculturally important. They are often classified as group A and group B compounds; group A trichothecenes are so classified due to the presence of a hydrogen or an ester group at the C-8 position (Meneely *et al.*, 2011). The most commonly reported group A trichothecenes include T-2 toxin, HT-2 toxin, neosolaniol, monoacetoxy scirpenol and diacetoxyscirpenol. Common group B trichothecenes are deoxynivalenol, nivalenol, 3- and 15- acetoxynivalenol and fusarenone X. Another group of trichothecenes are the macrocyclic trichothecenes produced by mould species such as *Stachybotrys atra* and include the satratoxins, verrucarins and roridins (EMAN, 2012).

The primary toxic effect of trichothecenes including DON is the inhibition of protein synthesis. Ingestion of DON by animals can also lead to acute gastrointestinal symptoms such as vomiting (hence its former common name of vomitoxin), feed refusal and bloody diarrhoea. The acute effects of DON in humans are similar to those in animals and involve adverse gastrointestinal symptoms such as nausea, vomiting, diarrhoea, abdominal pain, headache, dizziness and fever, which can appear 30 minutes after exposure. DON has been implicated in a number of incidents of human intoxication. In the period 1961 to 1985, about 35 outbreaks of acute foodborne illness were attributed to exposure to DON present in mouldy wheat and maize in China. In India in 1987, an outbreak of illness involving vomiting and dizziness among approximately 50,000 people was attributed to consumption of bread made from rain-damaged wheat that contained several trichothecenes including DON, with similar reports in Japan and Korea (Shephard, 2011). The interaction of DON with the immune system is complex and a number of mycotoxins, including the *Fusarium* toxins fumonisin, DON and T-2 toxin, have been suggested as potential risk factors for induction or persistence of chronic intestinal inflammatory diseases such as coeliac disease, Crohn's disease and ulcerative colitis. The evaluation of DON by IARC concluded that there was insufficient evidence for the carcinogenicity. It is also worth remembering that the phytotoxic nature of the moulds that produce the toxins as well as the toxins themselves may damage crop yields severely (Shephard, 2011). Cereals are the principle crop among many affected by the tricothecenes. Work in Europe reported in 2004 noted that typical DON concentrations (when found to be present above the limit of detection) in cereals were 2–250 µg kg^{-1} and, overall, tolerable daily human intake (TDI) was <1 µg per kg body weight. For infants and children however the data were very close to, or in some instances exceeded, the TDI (Meneely *et al.*, 2011).

The analytical chemistry of DON has received a great deal of attention. DON is most commonly extracted from cereals with methanol/water or and acetonitrile/water, although acidified acetone and ethyl acetate/acetonitrile/water have been reported to a lesser extent. The incorporation of water into the solvent encourages the grains to

swell, assisting the release of mycotoxins and tending to reduce extraction of interfering compounds. Clean-up now focuses on the use of solid-phase extraction (SPE), immunoaffinity columns (IACs) or ion-exchange columns rather than liquid–liquid partitioning. Screening assays using a variety of formats, principally ELISA but also including optical and electrochemical biosensors, have been published and are commercially available. Although gas chromatography has been applied for the analysis of DON, it requires laborious derivitization; liquid chromatography with fluorescence detection by pre-column derivitization is more popular. Liquid chromatography with (often tandem) mass spectrometry has been applied for definitive confirmation (Meneely *et al.*, 2011).

The UK Food Standards Agency has published (UK FSA, 2007) a Code of Good Agricultural Practice to reduce fusarium mycotoxins in cereals which emphasizes measures such as: avoiding maize as the previous crop; minimizing crop debris on soil surface; selecting resistant varieties; considering a spray application against fusarium ear blight; and timely harvesting.

Maximum concentrations of deoxynivalenol permitted by Regulation 1881/2006 (EC, 2006a) range from 500 μg kg^{-1} for bread and bakery ware and 750 μg kg^{-1} for dry pasta and cereals for direct human consumption and certain milling fractions of maize to 1750 μg kg^{-1} for unprocessed maize and durum wheat and oats. Processed cereal-based foods and baby foods for infants and young children must not contain more deoxynivalenol than 200 μg kg^{-1}.

8.3.1.5   Fumonisins

The fumonisins, compounds with three ether-linked carbon chain backbones with carboxyl, hydroxyl and an amino substituent(s), are mainly produced by *Fusarium* genera and are universal contaminants of maize and maize-based products. Identified in the mid-1980s there are at least 28 chemical analogues; the major fumonisins belong to the B series. Fumonisin B_1 (FB$_1$, empirical formula $C_{34}H_{59}NO_{15}$, relative molecular mass 721) is the most abundant (generally about 70% of the total fumonisin contamination) and normally co-occurs with lesser amounts of fumonisin B_2 (FB$_2$) and B_3 (FB$_3$). Chronic ingestion of fumonisins has been suggested as one possible risk factor for the occurrence of oesophageal cancer in South Africa, where fumonisin exposure from contaminated maize is high. Similar associations have been reported for maize in China, southern Brazil and in polenta produced in northern Italy. Fumonisins have also been linked as a risk factor for primary liver cancer in China, and the International Agency for Research on Cancer (IARC) has classified FB$_1$ as a possible human carcinogen. There is also epidemiological evidence and animal studies results indicating that fumonisins may have played a role in the elevated incidences of neural tube defects. Fumonisins are potent inhibitors of *de novo* sphingolipid biosynthesis; their depletion of sphingolipids interferes with the folate receptor, inhibiting uptake of folate, the cellular deficiency of which is a known cause of neural tube defects (Shephard, 2011).

Aqueous methanol or acetonitrile is used for extraction of fumonisins from maize and maize products with liquid chromatography of a fluorescent derivative (with p-anisaldehyde, fluorescamine or o-phthaldialdehyde) although ELISA is also commercially available. Confirmation with LC-MS/MS is also possible (Shephard, 2011; EMAN, 2012). Maximum concentrations of the sum of fumonisins B_1 and B_2 permitted by Regulation 1881/2006 range from 200 μg kg^{-1} for processed cereal-based foods and baby foods for infants and young children, 800 μg kg^{-1} for maize-based breakfast cereals and snacks, 1000 μg kg^{-1} for maize for direct human consumption and up to 4000 μg kg^{-1} for unprocessed maize.

8.3.1.6   T-2 and HT-2 toxin

T-2 toxin and HT-2 toxin are Type A trichothecene (see Section 8.3.1.4) mycotoxins produced by various *Fusarium* species which may occur on cereals and grasses in the temperate and cold areas of the world. HT-2 toxin is a hydrolysed (de-acetylated) naturally occurring analogue of T-2 toxin and is frequently analysed and evaluated together with T-2 toxin. T-2 toxin is one of

the most acute toxins of the trichothecenes. It is a potent inhibitor of protein, RNA and DNA synthesis and its toxic effects include digestive disorders, haemorrhage in many organs, oral lesions, dermatitis, leucopenia and blurred and painful vision. It has even been considered as a potential chemical weapon. Its acute effects in humans are most frequently described by a haemorrhagic syndrome, alimentary toxic aleukia (ATA). A similar effect in animals is referred to as mouldy corn toxicosis. It has been reported that ATA occurred in the former USSR during the first half of the 20th century and caused hundreds of thousands of deaths (Shephard, 2011).

Regulation 1881/2006 allows for the control of T-2 and HT-2 but as yet no numerical limits have been set in EU legislation. The Commission asked for further data on the presence of T-2 and HT-2 toxin in cereals and cereal products (not including rice) as well as other *Fusarium* toxins such as deoxynivalenol, zearalenone and fumonisin B_1 and B_2 to allow the extent of co-occurrence to be assessed. This was reinforced on March 2013 when the Commission published a Recommendation setting out indicative maxima for sum of T-2 and HT-2 above which investigations should be performed by Member States, with the active involvement of the feed and food business operators (especially in case of repetitive adverse findings). The indicative maxima for human consumption range from 15 µg kg^{-1} for cereal-based foods for infants and young children, 25 µg kg^{-1} for bread (including small bakery wares), pastries, biscuits, cereal snacks and pasta, up to 1000 µg kg^{-1} for oats (with husk). Indicative maxima are also given for animal feed (EC, 2013b). In Russia, specific maximum limits for T-2 toxin of 50–100 µg kg^{-1} are established for several foodstuffs (EMAN, 2012). Analytical methods have been discussed by Meneely *et al.* (2011).

8.3.1.7 Zearalenone

Zearalenone (ZON), a phenolic resorcylic acid lactone ($C_{18}H_{22}O_5$, 318.4 Da), is produced in cereals by e.g. *F. graminearum* and *F. culmorum* particularly during cool, wet growing and harvest seasons. It is not acutely toxic, but as a naturally occurring oestrogen exhibits strong hormonal properties, especially in the pig (Shephard, 2011; EMAN, 2012). For analysis, commonly used extraction solvents are aqueous mixtures of methanol, acetonitrile or ethyl acetate followed by a range of different clean-up procedures that depend in part on the food and on the detection method in use and include immunoaffinity columns. The analytical finish is by LC with UV or fluorescence detection, GC/ECD, GC-MS or LC-MS with limits of detection down to and below 5 µg kg^{-1}. ELISA can be used but can be less sensitive (EMAN, 2012). Regulation 1881/2006 sets out maxima for zearalenone from 20 µg kg^{-1} for processed maize-based foods for infants and young children, 50 µg kg^{-1} for bread, 75 µg kg^{-1} for cereals for direct human consumption, 100 µg kg^{-1} for maize-based breakfast cereals and snacks and up to 400 µg kg^{-1} for refined maize oil.

8.3.2 Aluminium in noodles

The adulteration of wheat flour and bread with alum (hydrated potassium aluminium sulphate) was a problem in 19th century Europe (Hassall, 1876). Aluminium in food was the focus of a great deal of attention for a time in the late 20th century when a link between aluminium intake (main source, food) and the development of Alzheimer's disease was postulated. Parenteral exposure to high aluminium concentrations by patients undergoing dialysis has led to neurotoxicity; however, oral intake of aluminium is no longer considered relevant to Alzheimer's disease. Nonetheless, food-derived aluminium intake appears to exceed desirable limits (EFSA, 2008). The question of aluminium in wheat and derived products was raised again in the 21st century when elevated concentrations of aluminium in imported noodles was discovered in Germany in November 2008 and confirmed by controls carried out by several other Member States. The concentrations of aluminium found ranged between 50 and 150 mg kg^{-1} (RASFF, 2009) and on 28 October 2009 Germany reported a RASFF, REF.2009.1412 recording aluminium concentrations of 265 mg kg^{-1} and 340 mg kg^{-1} in noodles

from Vietnam. These are higher than would generally be expected for naturally occurring concentrations of aluminium in foods (MAFF, 1997). As a consequence, EU law (Regulation No. 669/2009; EC, 2009a) on increased level of official controls on imports was amended by regulation (EU) No. 878/2010 of 6 October 2010 to require 10% of Chinese noodle imports to be analysed.

It has been speculated that the use of potassium aluminium sulphate, ammonium aluminium sulphate or other aluminium containing additives in noodles gave rise to the elevated findings noted above. However, it is also reported by the trade that Chinese authorities are taking stringent measures aimed at preventing the export of noodles with aluminium concentrations greater than 10 mg kg^{-1}.

8.3.2.1   Toxicology of aluminium

The human toxicology of aluminium from food, water and food contact materials was the subject of a comprehensive report in 2008 from EFSA (EFSA, 2008). The reader is referred to the full report for a considered view of the toxicology of aluminium but in brief, and echoing the Panel's view that in many important respects toxicological evidence is limited, the EFSA Panel concluded that aluminium is unlikely to be a human carcinogen at dietary relevant doses and that exposure to aluminium via food does not constitute a risk for developing Alzheimer's disease. The Panel noted that in animal studies several compounds containing aluminium have the potential to produce neurotoxicity and embryotoxicity, to affect the male reproductive system and to affect the developing nervous system in offspring. The Panel also noted that there are very few specific toxicological data for food additives containing aluminium; they therefore considered it prudent to take the adverse findings from animal studies into account when setting a tolerable intake for all dietary sources. For these and other detailed technical reasons, the EFSA Panel considered it appropriate (in 2008) to establish a tolerable weekly intake (TWI) for aluminium of 1 mg aluminium per kilogram body weight per week (1 mg aluminium kg^{-1} bw week^{-1}).

The TWI was reviewed again in 2011 by JECFA (2011) which established a provisional tolerable weekly intake (PTWI) of 2 mg kg^{-1} body weight based on a no-observed-adverse-effect level (NOAEL) of 30 mg kg^{-1} body weight per day and application of a safety factor of 100. This PTWI applies to all aluminium compounds in food, including food additives. The previous JECFA PTWI of 1 mg kg^{-1} body weight was withdrawn; however the EFSA TWI of 1 mg aluminium kg^{-1} bw week^{-1} remains the toxicological basis for risk assessment in the EU.

Both EFSA and JECFA consider that aluminium intake approached and may exceed TWIs. The EFSA Panel was aware that the TWI of 1 mg kg^{-1} bw week^{-1} is likely to be exceeded in a significant part of the European population. Cereals and cereal products, vegetables, beverages and certain infant formulae appear to be the main contributors to the European dietary aluminium exposure.

8.3.2.2   Current limits on aluminium in food in the EU

RASFF rejections of consignments of imported noodles are currently based on an aluminium limit of 10 mg kg^{-1} stemming from a view taken by the European Commission supported by the Standing Committee on the Food Chain and Animal Health (SCoFCAH, 2010). The relevant minute of the Standing Committee meeting reads as: 'Following requests for clarification as regards the proposed listing of noodles from China under Annex I to Regulation (EC) No 669/2009, the Commission clarified that, based upon available data, the level of 10 mg kg^{-1} could be used to distinguish noodles with acceptable unavoidable background presence of aluminium from noodles presenting unacceptable levels. Vote: qualified majority by 338 votes in favour, 7 votes abstained.' European Commission Regulation 380/2012, which came into force on 23rd May 2012, introduces restrictions on the use of aluminium-containing additives.

Aluminium is readily determined in food by a range of methods including microwave pressure acid digestion followed by inductively coupled plasma coupled with optical emission spectrometry or mass spectrometry (ICP-OES or ICP-MS). The expanded measurement uncertainty (MU) associated with such a determination in routine circumstances at around 10 mg kg^{-1} is about 15–20%. Much lower MU can be achieved, but at the increased cost associated with more, and multi-day, replicate analyses. Standard Reference Materials are readily available, containing certified concentrations of aluminium. One of these, along with appropriate blanks and spiked samples to provide matrix matched recovery checks and at appropriate replicates, are recommended for analytical batch runs. The authors consider that it is also important in regulatory work to report and have regard to the MU, basing regulatory decisions that depend on the criminal burden of proof on the mean result minus the MU when appraising a result against a limit (EC, 2004).

8.3.3   Veterinary residues: Nitrofurans

The six-month suspension of Bangladesh freshwater prawn consignments in 2009 due to the detection of the metabolites of banned nitrofuran antibiotics by Port Health laboratories in the EU, resulting in a loss of USD 350 million and the closure of many shrimp-processing plants, is referred to elsewhere in this work (*Achieving Quality Chemical Measurements in Foods*, Chapter 7). Nitrofuran veterinary antibiotics were widely used prior to being banned due to carcinogenicity. The parent drugs have a very

short half-life in the animal and so metabolite markers were developed to monitor their illicit use. Table 8.4 lists the compounds which are used as markers (Kennedy, 2002).

European law (Regulation 470/2009; EC, 2009b) provides that food of animal origin containing a nitrofuran metabolite such as one of the above fails to comply with Community legislation and is prohibited from entering the food supply chain, unless the concentration of such a metabolite does not equal or exceed a reference point for action pursuant to Regulation 470/2009. Such a reference point for action for nitrofuran metabolites has been established (Commission Decision 2003/181/EC; EC, 2003) as a minimum required performance limit (MRPL) of 1.0 µg kg^{-1}. Where the results of analysis confirm the presence of a nitrofuran metabolite below the reference point for action but above a decision limit, CCα determined as part of the analytical procedure (Commission Decision 2002/657/EC; EC, 2002b) an investigation is required on the part of the competent authority.

8.3.3.1   Analysis
The analysis of food for nitrofuran marker metabolites involves acid hydrolysis, the formation of nitrophenyl derivatives and LC-MS/MS, with isotopically labelled standards (Vass *et al.*, 2008).

8.3.3.2   SEM from sources other than nitrofurazone
The possibility that one of the marker metabolites, semicarbazide (SEM), may not arise simply as a metabolite of administered nitrofurazone was advanced in 2002. Kennedy (2002) reported that SEM could be detected in a range of materials

Table 8.4   Nitrofuran metabolite marker compounds

Parent drug	Marker metabolite	Abbreviation
Furazolidone	3-amino-oxazolidinone	AOZ
Furaltadone	3-amino-5-morpholinomethyl-1,3-oxazolidinone	AMOZ
Nitrofurantoine	1-aminohydantoin	AHD
Nitrofurazone	Semicarbazide	SEM

intended to coat chicken meat during the production of cooked chicken products. Most positive findings were associated with the use of breadcrumbs and other bread products. Subsequent investigations revealed that azodicarbonamide, a flour treatment agent that was commonly used in the production of certain breaded chicken products produced in Thailand, was the cause of the problem. This emphasized the importance of removing the coatings from chicken and shrimp products and of analysing the meat for the presence of bound residues of SEM. The use of azodicarbonamide as a food additive is not permitted in the EU. The EU Community Reference Laboratory issued the following advice on the confirmation of nitrofuran residues by analysis for SEM in December 2002 (CRL, 2002):

1. When testing composite food, only analyse the part of the product which is of animal origin, for example only the meat part of breaded products.
2. The detection of total (free + bound) residues of metabolites of nitrofurans can be maintained at the screening level.
3. In case of a non-compliant sample for total SEM, a sample must be reanalysed for the bound residues of SEM only. To this end, free SEM should be extracted/washed out prior to this confirmation test.

Azodicarbonamide is also known to be used as a blowing agent for plastic gaskets, and SEM was also thought to arise from carrageenan and through hypochlorite treatment of nitrogen-containing foods (Hoenicke *et al.*, 2004; EFSA, 2005).

Further, it has been shown that SEM may occur naturally in the shell of crustaceans including crabs, langoustines and shrimps. Although the metabolic route for its production in the animals is not confirmed, a role in protein synthesis is thought to be one of the possibilities. A solution is to analyse the inner core of the tested animal's meat, as SEM detected in wild-caught shrimp, presumably untreated, seems to be surface-associated (McCracken *et al.*, 2013).

8.3.4   Non-regulated contaminants

There are some contaminants for which legislation is not appropriate. Some compounds arise as artefacts of food processing or even cooking. It is difficult to regulate such compounds and the example of acrylamide will be briefly touched upon. Table 8.5 lists some contaminants other than those directly regulated in the EU. Further information is available on perfluoroalkinated organic substances at http://ec.europa.eu/food/food/chemicalsafety/contaminants/index_en.htm and at http://www.efsa.europa.eu/en/topics/topic/bfr.htm on brominated flame retardants. A brief update on phthalates is available from http://www.eatwellscotland.org/healthissues/factsbehindissues/phthalates/index.html.

8.3.4.1   Acrylamide

Acrylamide ($CH_2=CHCONH_2$, 71.1 Da), a well-known industrial chemical in the 20th century, has widespread uses particularly in the production of polymers and water and waste water treatment. In April 2002 however, the Swedish National Food Agency and Stockholm University revealed unexpected high concentrations of acrylamide in certain fried, baked and deep-fried foods, and later in coffee. Since acrylamide had been classified as a carcinogen by the International Agency for Research on Cancer (IARC), this caused widespread concern. It was also recognized that humans had probably been consuming acrylamide for centuries.

Table 8.5 Contaminants other than those regulated in the EU

Type	Contaminant
Arising from heat processing	Acrylamide; furans
Fermentation products	Ethyl carbamate
Industrial products	Perfluoroalkinated organic substances; brominated flame retardants; phthalates

Acrylamide may be formed in foods, typically carbohydrate-rich and low-protein plant commodities, during cooking or other thermal processing such as frying, baking or roasting at temperatures of 120°C or higher. Shortly after the unexpected findings of acrylamide in food were released, the EC Scientific Committee on Food (SCF) issued an opinion. The SCF expressed concerns about exposure levels and recommended that exposures should be as low as reasonably achievable (ALARA) (Dybing *et al.* 2005). Intensive research subsequently elucidated the reaction of reducing sugars with free asparagine as the main origin of acrylamide in food. In the context of the Maillard reaction, sugars and other carbonyl compounds play a specific role in the decarboxylation of asparagine as a necessary step in the generation of acrylamide (Yaylayan and Stadler, 2005).

The Confederation of the Food and Drink Industry (CIAA) and the *Codex Alimentarius* have separately developed (a) a 'toolbox' and (b) a Code of Practice respectively, containing measures that can be applied by different sectors of the food industry to reduce acrylamide levels. Sector-specific brochures have also been developed (Codex, 2009; FoodDrink Europe, 2011b). Monitoring by Member States is being collated by the Commission and EFSA and indicative values have been established ranging from 80 µg kg^{-1} for baby foods other than processed cereal based foods to 1000 µg kg^{-1} for potato crisps. It is emphasized that these indicative values are *not* safety thresholds but are intended only to indicate the need for an investigation if they are exceeded. The investigation should in particular look at the extent to which options for the minimization of acrylamide formation in the *Codex* Code of Practice and the CIAA acrylamide toolbox have been implemented by the food business operator (EC, 2011). Analytical methods such as gas chromatography-mass spectrometry (GC-MS) after bromination to 2,3-dibromopropanamide or direct determination with liquid chromatography-mass spectrometry (LC-MS) have been developed, although care needs to be taken to avoid artefact formation. These methods and subsequent studies on extraction and separation can be applied to both 'routine' and 'difficult' (i.e. chocolate and coffee) food matrices (IFST, 2013).

8.4 Resolution of disputed chemical results

EU Regulation 882/2004 harmonizes official controls on feed and food and allows businesses subject to those controls to have an analytical second opinion. UK law predates this safeguard by over 100 years. The regulatory landscape in the UK is a complex one, with policy set by central government and enforcement mainly a local government responsibility. Analysis by official food and feed control laboratories (the 'OCL system') provides the underpinning measurement science and chemical aspects of food and feed safety, while composition and labelling are dealt with by Public Analysts and Agricultural Analysts (generally the same official). Should an analytical dispute arise, a retained portion of the control sample may, in statutorily defined circumstances, be submitted as a technical appeal to the Government Chemist for a definitive investigation (i.e. 'referee analysis'). Walker and Gray (2013) described Government Chemist referee casework in the calendar years 2010 and 2011 and provided an opportunity to assess the performance of the technical appeal safeguard and the control system using the limited number of complex cases where appeal has been invoked.

The OCL system in the UK faced continuing funding challenges in 2010 and 2011 but generally performed well in areas where capability has been developed such as in aflatoxin analysis where Public Analysts' and Agricultural Analysts' findings were confirmed on technical appeal in 5/7 (71.4%) cases. Much more dispersion is evident in aflatoxin results between laboratories in animal feed samples than in food samples, however. Since largely the same laboratories are involved it is clear that sampling, and in particular the lack of a requirement that high shear mixing with water to form a slurry prior to splitting the samples into parts, is the main source of the variation. It is recommended that sampling

and sample preparation should be harmonized in the feed and food areas.

OCL performance was poorer in the more problematic area of drug residue analysis. Of six nitrofuran marker metabolite cases (all on imported crustaceans) only one (17%) was completely upheld. In 3 cases (50%) a residue was confirmed present, but at a concentration below the limit at which the consignment should be prohibited from entry into the UK. Research conducted in 2011 (Van Poucke *et al.* 2011; McCracken *et al.* 2013) demonstrated that the marker for nitrofurazone, semicarbazide SEM, is naturally occurring in crustacean shells. In two cases (33%) the Government Chemist confirmed SEM was not detected in the core flesh of the animals, overturning the OCL findings. The Government Chemist published a comprehensive advice note on nitrofuran analysis and since then the number of disputes has diminished with only one in 2012 in which the OCL findings were upheld. It is recommended that proposed better markers for nitrofurazone, such as its cyano derivative, should be further investigated and that the sampling of imported crustaceans should be reviewed to achieve better homogenization prior to sample subdivision. The diversity of measurement methods surveyed by Walker and Gray (2013) is evident and indeed represents only a fraction of the range of techniques OCLs and the Government Chemist must competently deploy to ensure the system is effective and responsive.

8.5   Conclusions and future outlook

Agri-food is a global business in the European Union (EU) which is, at least for now, the world's largest trader in food and drink. The European context in which food safety is regulated can be considered to impact on non-EU countries in two main ways: (a) an understanding of EU law and practice is essential in order to export food to the EU; and (b) as a model for other states seeking to satisfy consumers as they become more demanding and critical about their food choices.

Food and animal feed must be safe, authentic and properly labelled, the responsibility for which falls to those who make and sell it. There is, however, a public expectation of regulatory oversight by government; a complex system of laws and scientific surveillance has developed in Europe to safeguard consumers and the honest trader. For genotoxic carcinogens such as the aflatoxins and for compounds such as acrylamide which humankind has probably been ingesting for centuries, exposures should be as low as reasonably achievable (ALARA). This aim can be achieved either by strict maxima in legislation or by options for minimization of occurrence informed by industry good practice.

While this chapter cannot cover every contaminant, we have selected areas which are known to be problematic and selected references as far as possible from the open access literature so that further study can be followed up unhindered. We trust by these means to disseminate a better understanding of EU law and scientific practice so that scientists, regulators and traders may find a common basis to protect consumers and further scientific cooperation and global trade. Finally, we have presented the UK model in which the resolution of technical disputes on analysis or its interpretation are resolved by recourse to the Government Chemist and commend this model to other jurisdictions.

Acknowledgements

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9

Pesticide Residues in Food: Health Implications for Children and Women

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Summary

Primarily, chemical pesticides are applied with the objective of enhancing crop productivity by combating pests and vector-borne diseases in human beings. Due to the relatively low exposure of consumers to pesticide residues in foods, it is the opinion of the majority of health professionals involved in food safety that the risks of pesticide residues are far lower than risks from issues such as microbiological contamination, nutritional imbalance, environmental contaminants, and naturally occurring toxins. Still, the risks from pesticides in the diet are not zero. Examples of consumer poisoning from misapplication of pesticides have been documented, while pesticides may still pose theoretical risks from long-term exposure to consumers due to the scientific impossibility of proving otherwise.

9.1 Introduction

In the agriculture sector, chemical pesticides contribute significantly to increased yields of crops by controlling pests and insect-borne diseases such as malaria, dengue, encephalitis, filariasis, and others in human health. Nearly 2.5 million tons of pesticides are annually applied worldwide, an amount which has been steadily increasing with the passage of time (FAO, 2002). The need to increase world food production for the rapidly growing population is well recognized. One of the strategies to increase crop productivity is effective

pest management, as more than 45% of annual food production is lost by pest infestation. In tropical countries, crop losses are even more severe because the prevailing high temperature and humidity are highly conducive to rapid multiplication of the pests. The application of a wide variety of pesticides on crop plants is therefore necessary in the tropics to combat pests and vector-borne diseases. This may be one of the reasons for the high values of pesticides found in different crops, vegetables and other food commodities (Tariq, 2005). More than 500 different pesticide formulations are being used in this environment, mostly in the agriculture sector, although the control of biological public health hazards also continues to be an important field of application. At present, more than 108 types of insecticides, 30 types of fungicides, 39 types of weedicides, 5 types of acaricides, and 6 types of rodenticides are being used. The data indicate that about 80% of total pesticides are now being used on cotton plants; the remainder are applied to paddy, wheat, sugarcane, maize, fruits, vegetables, tobacco, and even to fodder crops (Azevedo, 1998).

9.2   Pesticides

9.2.1   Definition of pesticide

A pesticide is any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest (insects, mites, nematodes, weeds, rats, etc.), including insecticide, herbicide, fungicide, and various other substances used to control pests. The definition of pesticide varies with times and countries. However, the essence of pesticide remains basically constant: it is a (mixed) substance that is poisonous and efficient at targeting organisms and is safe to non-target organisms and environments (EPA, 2009).

9.2.2   History of pesticide production and application

The history of pesticides can be divided into three phases as follows.

1.   The first phase was an era of natural pesticides which started during the period before the 1870s. These were used in ancient Greece and included the application of sulfur.
2.   The second phase started in the 1870s and continued until 1945, referred to as the inorganic synthetic pesticides era. Both natural and inorganic materials were mainly used during this period.
3.   The third phase was an era of organic synthetic pesticides such as DDT, 2,4-D, and HCH, dieldrin, etc. which began in 1945 (Dhaliwal *et al.*, 2004).

Since then the majority of pesticides used are synthetic, referred to as chemical pesticides. The application of chemical pesticides, in particular organic synthesized pesticides, has been a significant mark of human civilization, which greatly protects and facilitates agricultural production. In the earlier period of organic synthesized pesticides, there were mainly three kinds of insecticides: carbamate, organophosphorus, and organochlorines. Soon after that, herbicides and fungicides achieved considerable development. The consumption of insecticides is anticipated to decline gradually, with the use of herbicides becoming more popular in the future. This trend may be observed in pesticide consumption worldwide (Xu, 1997).

9.2.3   Worldwide production and consumption of pesticides

Until the 1990s, sales of global pesticides remained relatively constant at between USD 270 and 300 billion of which 47% were herbicides, 79% insecticides, 19% fungicides/bactericides, and 5% the others. During the period 2007–2008, herbicides were ranked first in the three major categories of pesticides (insecticides, fungicides/bactericides, herbicides). Application of fungicides/bactericides increased rapidly and was ranked second. Europe is now the largest pesticide consumer in the world, followed by Asia. Most of the pesticides worldwide are used on fruit and vegetable crops. In the developed countries, herbicides are mostly used for maize. Since the 1980s, several pesticides have been developed including various biopesticides (Zhang *et al.*, 2011).

9.2.4 Benefits and risks of pesticide application

Worldwide, approximately 9,000 species of insects and mites, 50,000 species of plant pathogens, and 8,000 species of weeds damage crops. Insect pests cause an estimated 14% of loss, plant pathogens cause 13% loss, and weeds result in 13% loss (Pimentel, 2009a). Pesticides are so indispensable in agricultural production that about one-third of the agricultural products are produced by using pesticides (Liu *et al.*, 2002). Without the application of pesticide, the loss of fruits, vegetables, and cereals from pests would reach 78%, 54%, and 32%, respectively (Cooper and Dobson, 2007) and, when pesticides are used properly, losses of crops from pests decline to 35–42% (Pimentel, 1997; Liu and Liu, 1999).

In view of the world's limited croplands and growing population, it is necessary to take all measures to increase crop productivity in order to ensure food safety. It has been pointed out that if the consumption of pesticides was prohibited, food production in the USA would drop sharply and food prices would soar. Under such circumstances, the export of cotton, wheat, and soybean in the United States would decline by 27% and 132,000 jobs would be lost. Fungicides are used on 80% of fruit and vegetable crops in the United States. The economic value of the apple has increased to USD 1,223 million by using fungicides (Guo *et al.* 2007).

The risks of using pesticides are also serious, however (Pimentel, 2009b). Most pesticides are not spontaneously generated. Most of them are highly toxic to human beings and the environment. Pesticides and their degraded products flow into the atmosphere, soils, and rivers, resulting in the accumulation of toxic substances and thus threatening human health and the environment. The environmental pollution caused by pesticides in Asia, Africa, Latin America, the Middle East, and Eastern Europe is now serious. Even in earlier years the residuals of DDT, lindane, and dieldrin in fish, eggs and vegetables have been much beyond the safe range in India. In India the DDT content in the human body is the highest in the world (Zhang *et al.*, 2006, 2007; Zhang, 2008).

9.3 Pathway of pesticide residues in the food chain

Exposure to pesticides can occur via a number of pathways (e.g. food, drinking water, residential, occupational) and routes (oral, inhalation, dermal). Generally, each exposure pathway is the responsibility of the different departments or agencies within national governments or international bodies. Hence, assessment by each route is generally undertaken independently. However, it is the totality of exposure that determines risk, and this was recognized in the USA by the Food Quality Protection Act (FQPA) of 1996. This resulted in the requirement to include so-called aggregate exposure in the risk assessment of pesticides, i.e. exposure through multiple routes and multiple pathways. The contribution of a given route or pathway to overall exposure depends on the nature and type of the pesticide. For example, in US evaluation of *N*-methylcarbamates, exposures via food dominate, followed by residential exposure and then the drinking water. In contrast, with organophosphates residential exposure dominate, while drinking water is the dominant exposure for triazines (EPA, 2006, 2007). Under Council Directive 98/83/EC in Europe, the legally permitted limits have been established. These limits are based on analytical, not toxicological, considerations. Hence, even if several pesticides sharing a mode of action are present in drinking water, this should not be of toxicological concern. Other uses of a pesticide, such as a human or veterinary medicine, could contribute to overall exposure. Ideally, all such sources should be taken into account in the assessment. In practice however, relevant information on exposure by a number of these pathways is not available. Further work is required to develop methods to aggregate exposure via multiple pathways and sources. The US EPA has developed guidance that could form the basis of this (EPA, 2001).

9.3.1 Pesticide residues in soil and groundwater

The mobility of pesticides in soil and their bioavailability and transferability to other environmental compartments (the atmosphere, water

bodies) depends on the mechanisms and kinetics of their sorption and desorption from soil particles (Moorman *et al.* 2001).

An understanding of these processes is essential for transport modeling and the rational design of remedial measures against pollution (Struthers *et al.* 1998). It is recognized that the soil is also a potential pathway of pesticide transport to contaminate water, air, plants, food, and ultimately in humans via runoff and subsurface drainage; interflow and leaching; and the transfer of mineral nutrients and pesticides from soils into the plants and animals that constitute the human food chain (Figure 9.1).

The migration of agricultural pesticides to groundwater and surface water has become an issue of great concern as numerous incidents of contamination have been documented in developed countries. The treatment of water supplies

to remove pesticides is very expensive; protection of water resources from contamination is therefore a logical long-term option (Brown *et al.*, 1995). A fundamental contributor to the Green Revolution has been the development and application of pesticides for the control of a wide variety of insectivorous and herbaceous pests that would otherwise diminish the quantity and quality of food produce (Richardson, 1998). In the 1980s, traces of pesticides were detected in shallow drinking water wells where larger quantities of pesticides had been used (Ali and Jabbar, 1992; Ahad *et al.*, 2000, 2001; Tariq *et al.*, 2004) as well as in the surface waters (Parveen and Masud, 1988; Ahad *et al.*, 2006). Areas of cotton belt are particularly polluted (Ali and Jabbar, 1992; Ahad *et al.*, 2001; Tariq, 2005). In addition, ponding irrigation which is commonly practiced may result in faster water infiltration along with pesticides to
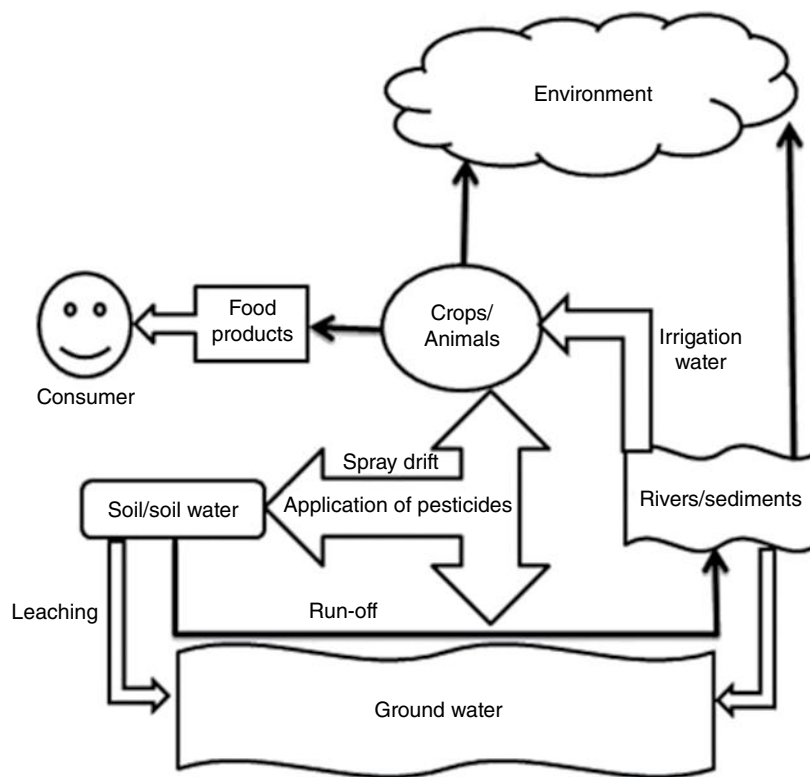
Figure 9.1   Possible pathways of a pesticide to food chain.

contaminate the groundwater (Flury *et al.*, 1994). Moreover, pesticides may reach the aquatic environment through direct runoff, leaching, careless disposal of empty containers, and washing of equipment, which resulted in approximately four-fold higher pyrethroid residue values in water and fish samples than EEC standards for drinking water (Tariq *et al.*, 2004; Ahad *et al.*, 2006).

9.3.2   Plant uptake of pesticide residues

Several researchers have reported on the ability of plants grown in soils containing bound residues to take up a portion of these residues. Plants have been found to take up bound residues of ^{14}C-trifuralin (Helling and Krivonak, 1978), ^{3}H-tri-fluralin [*methyl*-^{14}C] parathion (Fuhremann and Lichtenstein, 1978), ^{14}C-cypermethrin (Roberts and Standen, 1981), and ^{14}C-hydro-xymonolinuron (Hague *et al.*, 1982). Mustard plants took up small amounts of ^{14}C-monolinuron from soils which contained bound ^{14}C-monolinuron residues (Suss and Grampp, 1973). Corn plants could take up ^{14}C-residues from soil which contains bound ^{14}C-methabenzthia-zuron. In the majority of these studies, the proportion of the initial radioactivity found in plants fell within the range 1–5%. The residues are thought to exist as: (1) freely extractable residues; (2) extractable conjugates bound to natural components of plants; and (3) unextractable or bound residues incorporated into plant constituents, analogous to soil plant residues. These chemicals become part of the plant and transfer to leaves, roots, branches, stem, and plant produce. On consumption of plant parts, these chemicals are transferred to the end consumer and can have a lethal effect (Fuhr and Mittelstaedt, 1980).

9.3.3   Pesticide residues in feed and food

The effects of pesticides have been reported in milk, feed, cottonseed, different fruits, vegetables, and fish meal at different intervals and at different levels. A high variation of pesticide residues is noted in fruits and vegetables however, which may be due to changes in the climatic zone (hot, humid, and cold) and variation among different species of plants. However, the year-wise distribution of pesticide contaminations in fruit varieties (apples and citrus) has clearly shown that there is a marked reduction in the number of samples contaminated with pesticide residues as well as the number of samples exceeding maximum residue limits (MRL), assumed to be due to grower awareness of regulatory use of pesticides or otherwise (Munshi *et al.*, 2004; Parveen *et al.*, 2004; Saqib *et al.*, 2005).

Feed and fodder offered to animals are often contaminated with pesticide residues and, after feeding, these residues are assimilated into the body systems of the animals. The occurrence of pesticide residues in milk of ruminants is a matter of public health concern, since milk and dairy products are widely consumed by infants, children, and adults throughout the world. In view of this, many countries have enacted regulations to limit the level of pesticide residues in milk and dairy products (Prassad and Chhabra, 2001; Raikwar and Nag, 2003; Darko and Acquaah, 2008).

9.3.4   Pesticide residues in livestock/animal tissues

Livestock may be exposed to pesticide residues from contaminated feeds. Detection of chlorfluazuron (CFZ) residues in meat being processed in northern New South Wales in October 1994 resulted in the immediate quarantine of about 3000 beef farms and the temporary rejection of beef exports by Japan and the United States (Spence *et al.*, 1998). Investigations identified the source of the residues in cattle commodities to be from use of cotton gin-trash and cottonseed remnants as feeds during a prolonged drought in the early 1990s. The usual source of pesticide residues in feeds is through legitimate use of pesticides (herbicides, insecticides, and fungicides) in the commercial production of crops. Once ingested, lipophilic pesticides may be absorbed from the intestine to the systemic circulation via portal blood, and may be subject to metabolism

by the liver before entering systemic circulation. Chemicals with high lipid solubility tend to concentrate in tissues with higher fat content, such as adipose tissue, brain, liver, and kidney and, in the case of lactating animals, milk. The presence of a chemical in tissues and milk is also affected by its degree of biotransformation and its rate of elimination from the body. A continual problem for livestock industries is how to assess and manage trade-related risks associated with low levels of pesticide residues in meat (Pagliuca *et al.*, 2006; Sharma *et al.*, 2007; Nag and Raikwar, 2008).

9.4   Pesticide residue dissipation during processing

Aspects of the safety and quality of food products are the subject of ongoing debates and have caused concern in various segments of society. Scientific investigations are underway into better management of residues in food commercially available for consumption. For legislative regulations, the Codex Alimentarius Commission of the United Nation's Food and Agriculture Organization and the World Health Organization has established Maximum Residue Limits (MRLs) for pesticides in a variety of foods. More recently, there has been increasing interest among these agencies to obtain data on the fate of pesticide residues in processed food commodities (FAO, 2002). Food processing studies are designed to measure changes in residue levels when raw goods are converted to processed commodities (Abou-Arbab *et al.*, 1999b; Soliman, 2001; Anwar *et al.*, 2011). In general, it is accepted that food processing usually, but with some exceptions, results in a decline of residue level. Changes are simply expressed as processing factors (FAO, 2000):

$$\text{Processing factor} = \frac{\begin{array}{c}\text{Level of pesticide residue}\\\text{in processed commodity}\end{array}}{\begin{array}{c}\text{Level of pesticide residue}\\\text{in raw commodity}\end{array}}$$

9.4.1   Dissipation of pesticide residues by washing with water

Washing is the most common form of processing which is a preliminary step in both household and commercial preparations. Loosely held residues of several pesticides are removed with reasonable efficiency by the washing processes. Washing is generally the first step in various treatments which are given to food commodities. It can remove various types of pesticide residues from fruits and vegetables as summarized in Table 9.1. Generally, the pesticides which are systemic in action are difficult to remove during tap water washing.

9.4.2   Dissipation of pesticide residues by dipping in chemical solutions

Although tap water washing significantly removes broad types of pesticide residues from fruits and vegetables, several studies have indicated that the immersing of pesticide-contaminated fruits/vegetables in different chemical solutions is more helpful as compared to pesticide dissipation with tap water processing. In general, dipping in chemical solutions has a positive effect on pesticide reduction (Ahmad *et al.*, 2011). Generally, the dissipation of any particular pesticide increases with increased concentration of the chemical solution. Other factors affecting the dissipation of pesticides from vegetables during dipping operation are dipping time, agitation and temperature of the solution. The matrix effect is also another important parameter which affects dissipation of pesticide residues from vegetables (Table 9.2).

9.4.3   Dissipation of pesticide residues by heat treatment

Cooking is the act of preparing food for eating purposes by the application of heat. It encompasses a vast range of methods depending on customs and traditions, availability and the affordability of the resources. Literature is replete with work on the

Table 9.1 Dissipation of pesticide residues during washing (–: not detected).

Commodity	Pesticide	Residue dissipation (%)	Reference
	Phosalone	3	
Apple	Fenitrothion	14	Stepan *et al.* 2005
	Tolyfluanid	14	
	Diuron	78	
Olive	Terbuthylazine	82	Guardia-Rubio *et al.* 2006
	Endosulphan sulphate	0	
Tomato	Captan	80	Juraske *et al.* 2007
	Cypermethrin	49	
Okra	Deltamethrin	33	Zafar *et al.* 2012
	Cyhalothrin	22	
Bitter gourd	Endosulphan	59	Nath and Agnihotri 1984
	Dichlorvos		
Soybean	Malathion	80–90	Miyahara and Saito 1994
	Chlorpyrifos		
	Captan		
	Deltamethrin	90	
Spinach	Chlorpyrifos	79	
	3,5,6-trichloro-2-pyridinol	89	
	Endosulphan	78	
	Deltamethrin	68	
Cauliflower	Chlorpyrifos	73	
	3,5,6-trichloro-2-pyridinol	–	
	Endosulphan	85	
	Deltamethrin	74	
Potato	Chlorpyrifos	76	
	3,5,6-trichloro-2-pyridinol	–	
	Endosulphan	80	Randhawa *et al.* 2007a, b, 2008
	Deltamethrin	80	
Brinjal	Chlorpyrifos	82	
	3,5,6-trichloro-2-pyridinol	40	
	Endosulphan	90	
	Deltamethrin	65	
Tomato	Chlorpyrifos	75	
	3,5,6-trichloro-2-pyridinol	25	
	Endosulphan	75	
	Deltamethrin	85	
Okra	Chlorpyrifos	81	
	3,5,6-trichloro-2-pyridinol	50	
	Endosulphan	70	

effect of cooking on pesticide residues dissipation (Table 9.3). Generally, the more severe the heat treatment, the higher the dissipation of pesticide residues from foodstuffs. Sprayed pesticides tend to accumulate on the peels of fruits and vegetables. Hence, maximum dissipation of pesticide residues was observed after removing peels of fruits and vegetables, accompanied by suitable heat treatment (Stepan *et al.*, 2005). Cooking alone without the removal of skin or peel showed no remarkable results in this context (Nath and Agnihotri, 1984; Randhawa *et al.*, 2007a, 2007b, 2008). However,

Table 9.2   Dissipation of pesticide residues by dipping in chemical solutions

Commodity	Processing	Pesticide	Residue dissipation (%)	Reference
Okra	Washing with 8% acetic acid solution	Cypermethrin	47	
		Deltamethrin	55	
		Cyhalothrin	63	
Brinjal	Washing with 8% acetic acid solution	Cypermethrin	47	Zafar *et al.* 2012
		Deltamethrin	62	
		Cyhalothrin	46	
Cucumber	Washing with 9% acetic acid solution	Imidacloprid	82	
Bell Pepper	Washing with 9% acetic acid solution	Imidacloprid	68	Randhawa *et al.* 2013
Tomato		HCB	51.3	
		Lindane	47	
		p,p-DDT	33.7	Abou-Arbab 1999a
		Dimethoate	91.5	
		Profenofos	86	
		Pirimiphos-methyl	93.7	

Table 9.3   Dissipation of pesticide residues during heat treatment

Commodity	Processing	Pesticide	Residue dissipation (%)	Reference
Apples	Steam boiling followed by removal of peels	Phosalone	82	Stepan *et al.* 2005
		Fenitrothion	100	
		Tolyfluanid	100	
Dates	Dehydration	Dichlorvos (DDVP)	100	Banerjee *et al.* 2006
	Boiling (100°C/10 min)	Methyl parathion	68–74	Ahmed 2000
Milk	Pasteurization (63°C/30 min)	Methyl parathion	75–84	
		ETU	32	
Apricot	Sun drying	Bitertanol	50	Cabras *et al.* 1998
Grapes	Oven drying	Methamidophos	64.2–71.9	Athanasopoulos *et al.* 2005
Cherries	Canning	Tetrchlorvinphos	0.95	Fahey *et al.* 1970
Bitter gourd	Open cooking (10 min)	Endosulphan	0.6382	Nath and Agnihotri 1984

Commodity	Processing	Pesticide	Residue dissipation (%)	Reference
Spinach	Cooking	Deltamethrin	72	
		Chlorpyrifos	51	
		TCP	26	
Cauliflower	Cooking	Endosulphan	59	
		Deltamethrin	41	
		Chlorpyrifos	64	
		TCP	–	
Potato	Cooking	Endosulphan	64	
		Deltamethrin	13	
		Chlorpyrifos	14	
		TCP	–	
		Endosulphan	22	Randhawa *et al*.
Brinjal	Cooking	Deltamethrin	13	2007a, b, 2008
		Chlorpyrifos	10	
		TCP	–	
		Endosulphan	31	
Tomato	Cooking	Deltamethrin	45	
		Chlorpyrifos	53	
		TCP	–	
		Endosulphan	54	
Okra	Cooking	Deltamethrin	61	
		Chlorpyrifos	54	
		TCP	–	
		Endosulphan	46	

dehydration and canning showed satisfactory results (Fahey *et al.*, 1970; Banerjee *et al.*, 2006).

9.4.4   Dissipation of pesticide residues by low-temperature storage

The use of low temperature in order to extend the shelf life of food has a long history. Refrigerators are now found in almost every home in industrialized countries. Most fruits and vegetables are refrigerated to extend their freshness. Refrigeration also reduces the rate of reaction of enzymes and increases the availability of high-quality products to the consumers for extended periods of time. In addition, refrigeration also affects the level of pesticide residues during storage. Storing vegetables in a refrigerator at 4°C results in considerable dissipation of pesticide residues (Cengiz *et al.*

2006), whereas freezing only achieves a minor reduction in them (Abou-Arbab 1999a). A summary of dissipation of pesticide residues in foods stored at lower temperature with the passage of time is given in Table 9.4.

9.5   Pesticide residues in food and food products

9.5.1   Pesticide residues in fruits and vegetables

Despite the fact that pesticides are used with the aim of controlling insects, diseases, fungi, and other pests, they also leave behind trace amounts of residues on fruits and vegetables (Anwar *et al.* 2011). Pesticide application is an essential component of modern crop production technology however, and its use has been continuously increasing

Table 9.4 Dissipation of pesticide residues during cooling

Commodity	Processing	Pesticide	Residue dissipation (%)	Reference
Cucumber	Storage (3 days at 4°C)	DDVP	48.1	Cengiz et al. 2006
	Storage (6 days at 4°C)	DDVP	70.8	
		HCB Dimethoate	5.28	
		Lindine	7.02	
Tomatoes	Freezing	p,p-DDT	5.74	Abou-Arbab 1999a
		Profenofos	28.5	
		Pirimiphos methyl	26.6	

Table 9.5 Summary of different studies on pesticide residues in fruits and vegetables

Commodity	No. of samples	Pesticide detected	> MRL	Reference
Mango	18	Cypermethrin, methamedophos, monocrotophos, cyfluthrin, dialdrin, methyl parathian	–	Shah et al. 2007
Guava Plum Apricot Apple Okra	120	Cypermethrin, λcyhalothrin, fenvalerate, bifenthrin, deltamethrin, cyfluthrin, fenpropathrin, permethrin	–	Hussain and Siddique, 2010
Brinjal Spinach Cauliflower Tomato Potato	15 samples of each vegetable	Endosulphan	Tomato and brinjal exceeded MRL	Randhawa et al. 2007b

over the past decades. In Pakistan, pesticide applications are greatest on cotton crop followed by fruits and vegetables. Insecticides, herbicides, and fungicides are commonly used for crop protection throughout the country; insecticides are used the most. After a ban on the use of organochlorine insecticides in the 1980s (Parveen and Masud, 2003), pesticides such as organophosphates, synthetic pyrethroids and carbamates are now in use; these are less persistent and easily degraded in the environment. Organophosphate insecticide poisoning is a global health problem with approximately 3 million poisonings and 0.2 million deaths annually; these are irreversible inhibitors of acetylcholinesterase affecting the central nervous (CNS), cardiovascular, and reproductive systems, producing a wide range of effects (Aardema et al., 2008).

The current magnitude of translocation of vegetables grown with pesticides is seen as a danger to mankind, even in areas where very little pesticides are used. Table 9.5 summarizes the findings of different studies that detected pesticide residues in different fruits and vegetables. It highlights the fact that the pesticide problem does not merely concern the chemical industry, professional farmers, foresters and applicators, those concerned with protecting wildlife, or those responsible for control of malaria and other vector-borne diseases; rather, the pesticide problem concerns every person who wants food at a reasonable price and who wants his home free of vermin.

9.5.2   Pesticide residues in milk

Milk is essential for human health as it provides nearly all the nutrients needed by the body; however, milk is also considered as a source of excretion of some toxic compounds (pesticides) which are excreted by simple diffusion, if the feed is contaminated. All the chemical contaminants/substances present in the animal feed, regardless of their properties (i.e. basics, soluble or insoluble in organic solvents), can be transferred to milk through carry-over processes from animal feed intake (Klaassen *et al.* 1996). Organochlorine pesticide residues detected in milk fat samples procured from different countries are listed in Table 9.6. The table highlights that the highest values of pp'-DDE, α-HCH, β-HCH and γ-HCH were found in milk fat samples that were procured from Slovakia (Prachar *et al.*, 1995), Jordan (Salem *et al.*, 2009), India (Nag and Raikwar, 2008), and Mexico (Pardio *et al.*, 2003), respectively.

9.5.3   Pesticide residues in organic foods

The consumption of foods grown organically is often perceived to be of reduced risk due to reduced exposure to pesticide residues. Organic produce is grown without the use of synthetic agricultural products, including the most conventional pesticides (USDA, 2001). A study of 110 urban and suburban children found measurable levels of OP pesticide metabolites in the urine of all children sampled except for one child, whose parents reported buying exclusively organic produce. This finding suggested that conventionally grown produce might be a primary source of pesticide exposure for urban and suburban children (Lu *et al.*, 2001).

9.6   Pesticide residues in humans

Pesticide misuse has been implicated historically as a cause of human poisoning following consumption of foodstuffs contaminated with harmful residue levels. Several cases of poisoning have been reported, including one that resulted from the illegal application of carbamate insecticide to watermelons in California in 1985 that resulted in more than 1000 cases of human poisoning in the US and Canada (Ferrer and Cabral, 1991).

Pesticides are toxic to people's health and environment. Their sporadic use has been leading to significant consequences, not only to public health but also to food quality, resulting in an impact load on the environment and hence the development of pest resistance. There is considerable waste through overuse and misuse, adding to the cost and contributing to the adverse environmental and health consequences. Inappropriate application of pesticides affects the whole ecosystem by entering the residues in the food chain

Table 9.6   Organochlorine pesticide residues (mg/kg on fat basis) detected in milk fat in different countries

Country/location	pp'-DDE	α-HCH	β-HCH	γ-HCH	References
China/Beijing	0.038	0.024	0.011	0.012	Zhong *et al.* 2003
Ghana/Kumasi	0.001	n.a.	n.a.	n.d.	Darko & Acquaah 2008
Germany	0.007	0.010	0.006	0.015	Weigert *et al.* 1983
India/Bundelkhand	0.036	0.019	0.099	0.010	Nag & Raikwar 2008
India/Maharashtra	0.015	0.011	0.015	0.004	Pandit *et al.* 2002
Mexico/Medellin	0.039	0.013	0.023	0.049	
Mexico/Paso San Juan	0.018	0.013	0.017	0.022	Pardio *et al.* 2003
Mexico/Tlalixcoyn	0.024	0.031	0.069	0.128	
Slovakia/Bratislava	0.051	0.005	0.006	0.004	Prachar *et al.* 1995
Spain/León	0.005	n.a.	n.a.	0.003	Lozada *et al.* 1996
Jordan	0.027	0.060	0.073	n.d.	Salem *et al.* 2009

and polluting the soil, air, ground, and surface water. With increasing amounts used, concern about their adverse effects on non-target organisms, including human beings, has also grown. Non-target pesticide poisoning has been identified as the possible cause of fish killing, reproductive failure in birds, and illness in humans (Rao *et al.*, 1993). In fact, it has been estimated that less than 0.1% of the pesticide applied to crops actually reaches the target pest; the rest enters the environment gratuitously, contaminates soil, water, and air, where it can poison or otherwise adversely affect non-target organisms (Pimentel and Levitan, 1986). Furthermore, many pesticides can persist for long periods in an ecosystem; organochlorine insecticides, for instance, are still detectable in surface water 20 years after their use had been banned. Once a persistent pesticide has entered the food chain, it can undergo 'biomagnification', i.e., accumulation in the body tissues of organisms, where it may reach concentrations many times higher than in the surrounding environment. It is presumably because of the similarity of the threats posed to health and the environment that in US law the term 'pesticide' includes defoliants, desiccants, and plant growth regulators used for purposes other than pest control. Humans are exposed to pesticides (found in environmental media such as soil, water, air, and food) by different routes of exposure such as inhalation, ingestion, and dermal contact. This exposure to pesticides results in acute and chronic health problems. Pesticides being used in agricultural tracts are released into the environment and come into human contact directly or indirectly. This exposure to pesticides, both occupationally and environmentally, causes a range of human health problems (Abhilash and Singh, 2009).

The use of un-prescribed pesticides in inappropriate doses not only affects soil conditions but also destroys the healthy pool of bio-control agents that normally co-exist with the vegetation. These bio-control agents are the friends of agriculture and hence need to be nurtured, cared for, and developed by reducing reliance on the use of chemicals in agriculture. Through linear risk extrapolation of animal data and maximum exposure levels of 550 million people, it is reported that there are 37,000 cancer cases yearly associated with pesticide use in developing countries. Recently, it was reported that approximately 3 million people are poisoned and 200,000 die each year around the world from pesticide poisoning, the majority of which belong to developing countries (WHO, 1990; FAO, 2000). A major problem is that pesticides which developing countries have banned due to their toxic effects are still being used (Wilson and Tisdell, 2001). It is also believed that, in developing countries, the incidence of pesticide poisoning may be even greater due to under-reporting, lack of data, and misdiagnosis. Even in developed countries, despite the strict regulations and the use of safer pesticides, occupational exposures are significant (Tariq, 2005).

9.6.1   Pathways of pesticide residues in women

Exposure of woman to pesticides can occur via numerous exposure pathways, including household use of pesticide products, dietary exposure to pesticide residues, and exposure to agricultural drift. Biological monitoring studies indicate that pesticide exposures are widespread in pregnant women in New York City, Salinas Valley in California, the Netherlands, and Norway. These have also been detected in amniotic fluid, umbilical cord blood, meconium, and infant urine, indicating exposure of the fetus to pesticides (Berkowitz *et al.*, 2003; Bradman *et al.*, 2003, 2005; Whyatt *et al.*, 2003, 2009; Ye *et al.*, 2008, 2009; Ostrea *et al.* 2009).

The presence of persistent organochlorine (OC) pesticides in human serum may be explained by environmental exposure of the population. Foods are considered to represent a constant source of exposure, despite compliance with the maximum permitted residue levels (MRLs). According to a recent study in Barcelona (Spain), OC pesticides, the most persistent substances, appeared to have completely disappeared from some foodstuffs such as fruits, although they continued to be detected in other types of food such as vegetables and milk (Vicente *et al.* 2004). The presence and

persistence of OCs in our bodies is a constant feature, whatever the source of exposure. They have been universally reported in human fatty tissue and serum, although most studies are based on serum levels given the ease of access to this medium. Numerous studies have related the presence of OCs in human samples to the consumption of certain foods, especially fish (Cole *et al.*, 2002; Chun and Kang, 2003; Jiang *et al.*, 2005). Infants can also ingest OCs in breast milk from chemicals accumulated in the fatty tissue of the mother during her life (Schafer and Kegly, 2002; Glynn *et al.*, 2003; Botella *et al.*, 2004; Minh *et al.*, 2004; De-Saeger *et al.*, 2005).

9.6.2 Pathways of pesticide residues in children

The most vulnerable periods for toxic impact of environmental pollutants on human development are the embryonic and foetal stages (Selevan *et al.*, 2000; Weiss, 2000; Daston *et al.*, 2004). Pregnant and nursing women pass these pollutants to their babies both transplacentally and via lactation, and evaluating the maternal contamination is an indirect measurement of the exposure of the foetus to external contaminants (Wang *et al.*, 2004; Suzuki *et al.*, 2005). One of the most significant concerns regarding health effects is the harmful influence of polychlorinated biphenyls (PCBs), polychlorinated dibenzoparadioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) on future generations, stemming from prenatal and/or postnatal exposure (Wang *et al.*, 2004).

Dietary ingestion is one of the pathways by which children are exposed to pesticide (Berry, 1997; Thomas *et al.*, 1997; ILSI, 1999; Akland *et al.*, 2000). Children eat more food per body mass than adults, and their diets differ from those of adults. These diets are often rich in foods containing higher levels of pesticide residues such as juices, fresh fruits, and fresh vegetables (National Research Council, 1993). Several national programs monitor pesticide levels in the food supply (FDA, 1996; USDA, 1997). The toxicity of pesticides in infants and children may

differ quantitatively and qualitatively from adults. Considering the multitude of risks associated with pesticide intake by infants, the European Union has set a very low limit for pesticides in infant food. According to this regulation, infant formulae must not contain residues of individual pesticides at levels exceeding 0.01 mg/kg (MRL) (Carmen *et al.*, 2009).

9.7 Health repercussions

The occurrence of residues of pesticides in food commodities is a key issue in public health. When exposed to human beings, these residues solicit a different response at the target depending on whether the target is a man or woman. The selected responses of pesticide exposure on reproductive health of either men or women are listed in the Table 9.7 while Table 9.8 summarizes the adverse reproductive health effects of pesticide formulations on females. Exposure of men or women to certain pesticides at sufficient doses may increase the risk for sperm abnormalities, decreased fertility, a deficit of male children, spontaneous abortion, birth defects or fetal growth retardation. Pesticides from workplace or environmental exposures enter breast milk. Certain pesticides have been linked to developmental neurobehavioral problems, altered function of immune cells, and possibly childhood leukemia. In well-designed epidemiological studies, adverse reproductive or developmental effects have been associated with mixed pesticide exposure in occupational settings, particularly when personal protective equipment is not used. Every class of pesticides has at least one agent capable of affecting a reproductive or developmental endpoint in laboratory animals or people, including organophosphates, carbamates, pyrethroids, herbicides, fungicides, fumigants, and especially organochlorines. It has also been observed that pesticide exposures are increasingly linked to immune suppression, hormone disruption, diminished intelligence, and reproductive abnormalities. Many pesticides and their residues are known to be contributory factors in

Table 9.7   Selected reproductive health problems after exposure to pesticides among men or women

Sr. No.	Problem	Partner exposed
1.	Reduced fertility	Woman, man
2.	Abnormal sperm genetic material or indices	Man
3.	Deficit of male children	Man
4.	Spontaneous abortion	Woman, man
5.	Birth defects	Woman, man
6.	Fetal growth retardation, preterm birth	Woman
7.	Breast milk contamination	Woman
8.	Childhood neurobehavioral problems	Woman
9.	Developmental immunotoxicity	Woman
10.	Childhood cancer	Possibly woman

Table 9.8   Adverse reproductive health effects on females associated with pesticide formulations

Sr. No.	Pesticide class	Example agents	Study details	Reference
1.	Mixed exposure	Wide variety	Longer time to pregnancy among women whose spouses work in greenhouses. Spontaneous abortions in wives and birth defects in children of farmers or pesticide applicators. Women with infertility more likely than controls to be exposed to pesticides. Risk of childhood leukemia after indoor pesticide exposure during pregnancy. Formulations may include organic solvents that exhibit reproductive or developmental toxicity.	Ngo *et al.* 2010
2.	Organo-phosphates	Chlorpyrifos, diazinon, malathion, parathion	Lower birth weight and shorter gestational age among women with residential or agricultural exposure (agents listed on left). Neurodevelopmental or childhood behavioral problems (chlorpyrifos diazinon). Altered fetal immune cell function in rats (chlorpyrifos).	Kamanyire and Karalliedde 2004
3.	Carbamates	Carbaryl, propoxur	Neurodevelopmental or childhood behavioral problems (carbaryl). Possibly childhood leukemia (propoxur).	Lifshitz *et al.* 1999
4.	Pyrethroids	Deltamethrin, permethrin	Neurodevelopmental or childhood behavioral problems (deltamethrin). Possibly childhood leukemia (very high exposure to permethrin).	Cantalamessa 1993
5.	Herbicides	Chlorophenoxy herbicides, triazines, glyphosate	Women with infertility more likely to mix and apply herbicides. Spontaneous abortions in farm families using certain herbicides. Limb and heart defects in children of pesticide applicators oragricultural workers using herbicides. Early pregnancy loss and altered fetal immune cell function in rats; possibly human fetal growth restriction from community drinking water (atrazine).	Ashby *et al.* 1996

Sr. No.	Pesticide class	Example agents	Study details	Reference
6.	Fungicides	Vinclozolin, thiram	Women with infertility more likely to use fungicides supported by some studies in mice and rats. Limb defects in children of pesticide applicators using fungicides. Pregnant rats exposed during gonadal sex determination had male offspring with decreased sperm concentration that also affected subsequent generations of males (vinclozolin).	Dalvie *et al.* 2009
7.	Fumigants	Aluminium phosphide, sulfuryl fluoride	Significant phosphine-induced inhibition of red blood cell cholinesterase occurs at concentrations of phosphine exceeding 10 µg/ml.	Potter *et al.* 1991
			Sulfuryl fluoride cause neurotoxicology in rats	Hanley *et al.* 1989
8.	Organo-chlorines	Dioxin, methoxychlor, DDT, others	Increased rates of endometriosis among women in Seveso Italy (dioxin) supported by studies in mice rats and monkeys. Fetal growth restriction in women with diets high in contaminated fish or who have higher blood levels of chlorinated pesticides (DDT and others). Pregnant rats exposed during gonadal sex determination had male offspring with decreased sperm concentration that also affected subsequent generations of males (methoxychlor). Decreased immune cell function at birth and increased middle ear infections after human in utero exposure.	Mishra and Sharma 2011

heart, Alzeheimer's and Parkinson's diseases. The increasing incidence of cancer, chronic kidney diseases, sterility among males and females, endocrine disorders, neurological and behavioral disorders, especially among children and women, have been attributed to chronic pesticide poisoning. Moderate human health hazards from the misapplication of pesticides include mild headaches, flu, skin rashes, and blurred vision; other neurological disorders, while rare but severe, include paralysis, blindness, and even death. Pesticide pollution to the local environment also affects the lives of birds, wildlife, domestic animals, fish, and livestock (Abhilash & Singh, 2009).

When women are sufficiently exposed to certain pesticides, several types of adverse reproductive outcomes may occur and developmental problems in their children may result. In addition to the relatively low levels of environmental exposure from sources such as fish, fruit, and vegetables, women can be occupationally exposed to large doses of pesticides as applicators, farm workers, and in other jobs. In the Agricultural Health Study, 1359 female licensed pesticide applicators were enrolled from just two states (North Carolina and Iowa). In developing countries, it is common for women to perform chemically intensive agricultural tasks which may cause adverse health effects after exposure, as mentioned earlier in Table 9.7.

9.8 Measures to combat pesticide exposure

Many of the most toxic pesticides have been banned or restricted in developed nations, but high exposures to these agents are still occurring in developing countries around the globe. Protective clothing, masks, and gloves are more

difficult to tolerate in hot and humid weather, or may be unavailable or unaffordable. Advising patients who are concerned about the reproductive and developmental effects of pesticides often involves helping them to assess their exposure levels, weigh risks and benefits, and adopt practices to reduce or eliminate their absorbed dose. Patients may not realize that by the first prenatal care visit, most disruptions of organogenesis have already occurred. Planning ahead provides the best chance of lowering risk from pesticides and remediating other risk factors before conception.

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10

The Need for a Closer Look at Pesticide Toxicity during GMO Assessment

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Summary

Public policy is regularly shaken by health crises or unexpected discoveries; future directions in toxicology assessment are therefore urgently needed. This chapter focuses on agricultural genetically modified organisms (GMOs) because they are essentially pesticide-plants, designed to tolerate and/or produce new pesticide residues in food and feed. Moreover, the usual concepts of regulatory toxicology become erroneous or insufficient with regards to endocrine or nervous disruption, or epigenetic effects. Most pollutants affect cell-cell communication systems in the same way as unsolicited spam emails, and may promote chronic and environmental pathologies. We also describe how formulated pesticides are mixtures which have not been investigated for their long-term toxicities. They contain adjuvants that are even more toxic than the supposedly active principle. Finally, long-term and multigenerational testing *in vivo* often appears essential. This can be accomplished within two years on rats, independently of the biotechnology industry, with raw data being transparent to the scientific community to allow healthy debate before the next health crisis.

10.1   Purpose, aim and scope

In the matter of health issues, public policy is regularly shaken by health crises or unexpected scientific discoveries. Late lessons are learned from early warnings of toxicity of pesticides and pollutants in general (European Environment Agency, 2013). There is an inescapable lag between scientific discoveries and advances in regulation, but this may be amplified by private, political, or economic interests. History has taught us that environmental hazards could be avoided by proper risk assessment and the use of active preventive research, according to the precautionary principle (European Environment Agency, 2013). Unfortunately, several decades ago this was not the case for health risk management of thalidomide, diethystilbestrol, asbestos, organochlorine pesticides, plasticizers, and heavy metals, which were all believed to be safe and used at a large scale. These substances were regulated after several crises, and sometimes only prohibited or limited in their use after decades of contradictory debates as was the case for the ban on tobacco smoking in public areas or the proper labeling of risks. The lack of scientific knowledge in these matters had huge costs for public health and past, present, and future issues of practical food safety. It is now time to study this challenge for food and feed based on genetically modified organisms (GMOs) and the specific pesticides used or produced during their growth (see in particular Section 10.3 on pesticide plants).

As one of the research teams who published the most on the toxicity of edible GMOs and their associated pesticides, we were involved in an international scientific maelstrom when we published the first lifelong toxicological assessment in mammals of a Roundup-tolerant GM maize (NK603) and of the whole Roundup formulation (Séralini *et al.*, 2013). This GM maize was designed to tolerate (and thus to contain without dying) the Roundup formulation. Almost all agricultural GMOs cultivated at a large scale are pesticide plants, accumulating unusual levels of pesticide residues. Our long-term test was similarly criticized by health agencies and the biotech industry promoting the commercial authorizations of these products without conducting lifelong tests. We carefully answered all critics (Séralini *et al.*, 2013).

In particular, we noticed that pesticide toxicity was overlooked in these files. For instance, the modified insecticidal *Bt* toxins, known to be produced by *Bt* maize or cotton, were never tested on human cells between 1995 (origin of these commercial GMOs) and 2012, when our group tested them (Mesnage *et al.*, 2012a). By contrast, the main toxicological assessment of these GMOs is the establishment of the substantial equivalence (relative to gross chemical composition) with their natural counterparts or the closest isogenic lines of plants for controls. It has become obvious that more rigor in GM research is necessary (Domingo, 2000; Graef *et al.*, 2012; Séralini *et al.*, 2009, 2011; Spiroux de Vendômois *et al.*, 2010). This is true in particular on their potential toxicities as pesticide carriers (the reason why they were genetically modified), or due to unexpected genetic or metabolic disruptions caused by the technology itself.

10.2   A silent pandemic

Pesticides alone in non-GM treated plants, in the food chain or in the environment are already believed to be responsible for a silent pandemic, as described in Sections 10.2.1 and 10.2.2.

10.2.1   First observations on animal and human reproduction

Pesticides are formulated toxics, supposed to be specific to plants (herbicides), insects (insecticides), or fungi (fungicides). However, non-target effects are very often described in the literature (Colborn *et al.*, 1993; Androutsopoulos *et al.*, 2012; Mrema *et al.*, 2012) because they act as disruptors of the universal cellular metabolism (quite often on the respiration chain), or disruptors of cell–cell communications highlighted by hormonal or nervous disruption *in vivo*. Pesticides are widely used, not only in agriculture but also in the environment (public or private

parks, gardens, along roads and railway tracks, etc.) or even indoors for domestic use (insecticides, acaricides) or medical use (human head lice products).

These products have been used on a large scale for decades, and about GBP 5.2 billion are used worldwide every year (US EPA, 2012). Their spraying was intense after the Second World War, until it was noticed how detrimental they were to wildlife and then in mankind (Carlsen *et al.*, 1992; Colborn *et al.*, 1993). One of the first people to sound the alarm was Rachel Carson with her book *Silent Spring* (Carson, 1962) which was widely read.

It is noteworthy that early warnings concentrated mostly on the reproduction problems of wild populations of animals. An important remark is that the physiological function of reproduction initially appears more sensitive to environmental pollutions, rather than other physiological vital functions maintaining the homeostasis of the body, for instance blood composition and circulation, heartbeat, respiration, digestion, or elimination. Reproduction is non-essential for the individual life, but essential for the survival of the population as a whole. It is therefore one of the first phenomena to be affected by a physiological stress of an individual. Moreover, the meiosis at the origin of sexual cells and pregnancy are fragile phenomena which are very sensitive to environmental toxics. It also demands more energy than basic physiology. It is therefore understandable that early warnings discovered on the effects of pollutants in wildlife always concerned reproductive problems in different species.

One of the first to be reported was in 1952, when the ornithologist Charles Broley noticed a change in the behavior of bald eagles in Florida and discovered abandoned nests. Eighty percent of the birds appeared to be sterile and the number of juveniles declined (Broley, 1958). This symbolic eagle associated with the flag or US seal had an increase in blood levels of organochlorine pesticides (Coon *et al.*, 1970). Alligators of Lake Apopka, near a factory releasing large amounts of dicofol and DDT, developed an atrophy of the penis which led to the decline of the population (Guillette *et al.*, 1994). The reproduction of Florida panthers was impaired when male panthers became feminized as a result of prenatal or postnatal exposure to endocrine-disrupting chemicals such PCBs and pesticides such as DDT or methoxychlor (Facemire *et al.*, 1995).

Male reproductive health was also affected in the meantime. A decline in sperm quantity and quality was first noticed by Carlsen *et al.* (1992) and later confirmed by Jorgensen *et al.* (2001). A rising incidence of testicular cancer (Jacobsen *et al.*, 2000) and an increase in birth defects such as hypospadias and cryptorchidism was observed and grouped under the term 'testicular dysgenesis syndrome' by Niels Shakkebaek (Skakkebaek *et al.*, 2001).

10.2.2 Endocrine and nervous disruptions due to the aromatic structure of pesticides

Pesticides and plasticizers as well as large number of industrial products (paints, inks, etc.) are often synthesized from petroleum chemistry. The fossilization of plant aromatic compounds takes millions of years of sedimentation to make aromatic stable petroleum compounds. Plant aromatic compounds help sexual plant reproduction by attracting insects, and also have pheromonal activities for mammals in perfumes since they structurally resemble animal sex steroids with aromatic cycles. They are in general part of sexual signaling systems. The petroleum molecules chemically processed and pesticides have for this reason aromatic or pseudo-aromatic structures (Figure 10.1), like plasticizers. For instance, phytoestrogens are structurally and physiologically close to animal estrogens. This is why we can observe a crossed reactivity between these cyclic compounds in plants or petroleum and steroid hormones. Their endocrine-disrupting activities, including pesticides, are hardly surprising, and may be at the origin of reproductive disorders in the last 50 years since large stable aromatic compounds began to be spread in the environment by industry.

Most pesticides are usually stable and lipophilic because of their petroleum origin. As a consequence, they persist in fat tissues and can bioaccumulate in organisms, increasing their levels in the food chain
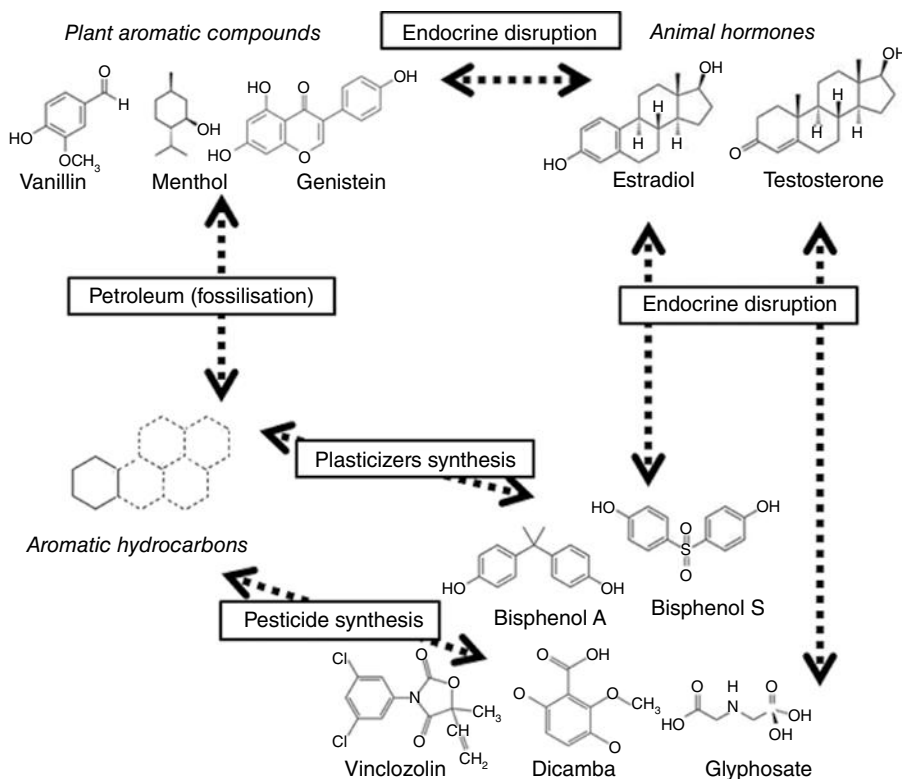
Figure 10.1   The origin of pesticides and plasticizers from petroleum and plant aromatic compounds. The fossilization of plant aromatic compounds, in particular, takes millions of years of sedimentation to make aromatic stable petroleum compounds. Plant aromatic compounds structurally resemble animal sex steroids with aromatic cycles, and this explains their sexual effects and pheromonal activities. For this reason, chemically processed petroleum molecules and pesticides have aromatic or pseudo-aromatic structures, like plasticizers. This is why we can observe a crossed reactivity between these cyclic compounds in plants or petroleum and steroid hormones.

cycle (Smith, 1999). Moreover, breastfeeding allows the transfer of xenobiotics ingested from the mother to the baby (Bouwman *et al.*, 2012). As a consequence, xenobiotics or their effects can be transmitted from one generation to the next.

Their lipophilicity also makes them act as electric insulators. This has been particularly researched for plasticizers surrounding food or feed, to prevent microbiological organisms from breaking in. This is particularly a concern for plastic baby bottles that allow the contamination of infants by plasticizers (Kubwabo *et al.*, 2009). Pesticides can also modulate neuronal activity however because the brain, physiologically dependent on neurosteroids, is very sensitive to aromatic compounds such

as estrogen, catechol-estrogen or catecholamines (Gonzales *et al.*, 2007). They may even modulate sexual behavior (Balthazart *et al.*, 1990) and can also initiate Parkinson's disease (Gaikwad *et al.*, 2011). In some cases, pesticides trigger neurological impairment (Rosenstock *et al.*, 1991; Rohlman *et al.*, 2007). Alzheimer's or Parkinson's neurodegenerative diseases are observed after both chronic and acute intoxication by pesticides (Elbaz *et al.*, 2009; Weisskopf *et al.*, 2010; Parron *et al.*, 2011).

All these links make it possible to understand the endocrine or nervous disruptions, and in general cell–cell communications interactions, linked to the general structure of aromatic pesticides resembling hormones.

10.3   Link between pesticides and agricultural GMOs

Here we describe more precisely the links between agricultural GMOs and pesticides. The application of genetic engineering in agricultural practices was advocated as the most important recent advance in plant protection for the last decades (Van Montagu, 2011). The industry claimed to reduce the use of pesticides by introducing genetically modified (GM) plants (Monsanto, 2013). This is not yet established however, since an increase, in particular in Roundup use, is documented (Benbrook, 2012). However, it is clear after 15 years that pesticides are the keystone of the agricultural GM characters. Almost all commercially cultivated agricultural GMOs are pesticide plants since their origin. For instance in 2011, 160 million hectares of GM crops were cultivated worldwide, with 59% of herbicide tolerance only (mainly Roundup), mostly in soybean, maize, canola, and cotton. They promote the use of the main herbicide of the world, Roundup, on edible plants. Another 15% of GM crops had for their GM character insecticide production only; 26% had stacked traits combining both characters (James, 2011). For example, SmartStax maize has six traits of different insecticide production (*Bt*) and two traits for herbicide tolerance, and therefore may contain up to 8 kinds of pesticide residues in addition to those which can be conventionally applied such as fungicides.

This method of pest management was agronomically criticized. These GM crops were first promoted as new tools for Integrated Pest Management (IPM). However, these practices cannot fulfill the main ecological principle of IPM, generally considered as a protection measure which should be synchronized only when pest damage exceeds a critical level (Székács and Darvas, 2013). In fact, the cultivation of GM plants is necessarily accompanied by the intensive use of glyphosate-based herbicides, and even more so when glyphosate-resistant weeds develop (Owen, 2008). This is amplified by the systematic insecticidal production at high levels in *Bt* plants. However the use of additional insecticide sprays

(Tabashnik *et al.*, 2008) increased, explained by a raise in insect resistances (Devos *et al.*, 2012).

For the cultivation of GM plants, Roundup may be sprayed several times in almost 80% of cases during the plant growth, simply because the plant can tolerate it. In fact, Roundup kills almost all normal plants except GM plants, and this facilitates the growth and harvest of some GM crops. GM plants therefore accumulate Roundup residues during their growth, while residue contents increase with the number of sprays (Arregui *et al.*, 2004). Because of the high content of GMOs in these residues, the Maximum Residue Limits (MRL) of glyphosate and aminomethylphosphonic acid (AMPA) (a compound of Roundup and its main metabolite used as markers) have been considerably increased with the development of these GMOs. MRLs represent the maximum level of residues expected when applying a pesticide according to 'good' agricultural practices. When other pesticides are generally found in edible plants at levels around 0.01–0.1 ppb (Sanco, 2013), glyphosate and its metabolite AMPA have among the highest MRL levels with up to 500 ppm authorized in GM feed (Sanco, 2013). Even 2 ppm of glyphosate and AMPA were authorized in bovine kidney, for instance (EFSA, 2009), since cattle are increasingly fed with transgenic Roundup-tolerant soya. The MRL in transgenic soybean, a major edible GMO grown for farm animals, has been set to 20 ppm.

For the second character, around 20 ppm of *Bt* toxin can persist in the transgenic plant (Székács *et al.*, 2010). This content can differ according to the GM variety and environmental conditions (Then and Lorch, 2008). Note that standardized guidelines to assay *Bt* toxins that are reproducible are lacking (Székács *et al.*, 2011). Herbicide-tolerant plants and *Bt* corns are regularly consumed not only by mammals but also by humans (on a regular basis in America), and their residues are even found in maternal and fetal cord serum (up to 93 ppb of glyphosate and around 0.2 ppb for *Bt* residue; Aris and Leblanc, 2011), but this does not take into account the possible bioaccumulation in the tissues. Roundup residues are not only found

in the urine of farmers spraying glyphosate-based herbicides but also in the urine of their children living far from the fields (Mesnage *et al.*, 2012b).

We consider as pesticides in GM plants not only the new modified *Bt* toxins alone or in combination, but also the herbicide residues that they tolerate, mostly glyphosate, with the adjuvants that go with these. As a matter of fact, the active principle of a pesticide is never used alone. Its toxicity is technically amplified by the choice of adjuvants, as cell penetrant helpers and stabilizers (Baynes and Riviere, 1998; Marutani and Edirveerasingam, 2006). Some of these, such as polyethoxylated (15) tallowamine (POE-15), are even more toxic on human cells than glyphosate (Mesnage *et al.*, 2013).

A minority of GM plants may also tolerate other herbicides such as glufosinate, bromoxynil, or the most recent ones such as 2,4-D and dicamba. Gufosinate ammonium is structurally related to glutamate which acts in neurotransmission in mammals. This other GM pesticide can induce spatial memory impairments, hippocampal magnetic resonance imaging modifications, and glutamine synthetase activation after chronic exposures (Calas *et al.*, 2008). Bromoxynil disrupts the development of the axial skeleton in mammals (Chernoff *et al.*, 1991; Kawanishi *et al.*, 2003). The 2,4-dichlorophenoxyacetic acid (2,4-D), a major ingredient of Agent Orange, is a synthetic auxin, a class of plant hormones. Its effects on health vary from a suspected carcinogenicity (Zahm *et al.*, 1990) to developmental effects such as a higher rate of morphologic and skeletal abnormalities in fetuses exposed during pregnancy to 2,4-D (Mazhar *et al.*, 2012). Dicamba is known as being genotoxic (Gonzalez *et al.*, 2009), but its role in the incidence of some cancers is debated (Weichenthal *et al.*, 2010).

10.4   Focus on Roundup toxicity in GMOs

The regulation of GMOs and the study of their health effects as pesticide plants is a hot topic of research.

10.4.1   Adjuvants: glyphosate is not the major toxicant in Roundup

Glyphosate is the most-used herbicide active ingredient in commercial formulations of pesticides worldwide, including insecticides and fungicides. Its formulations are made up of 36–48% of glyphosate, water, salts, and adjuvants such as polyethoxylated tallow amines or polyethoxylated etheralkylamine (POEAs), isobutane, sodium benzoate, sodium salt of o-phenylphenol, light petroleum distillate, methyl p-hydroxybenzoate, and 5-chloro-2-methyl 3(2H)-isothiazolone (Cox, 2004).

We have tested more than 10 glyphosate-based formulations on 10 mammalian cell types and 3 microorganisms. These included placental cell lines and fresh placenta (Richard *et al.*, 2005), embryonic human kidney cell lines HEK293 (Benachour *et al.*, 2007), umbilical cord primary cells (Benachour and Séralini, 2009), hepatic cell lines HepG2 and Hep3B (Gasnier *et al.*, 2010, 2011) and freshly isolated testicular cells (Leydig, Sertoli, germ cells, cocultures of Sertoli and germ cells; Clair *et al.*, 2012b), and 3 milk microorganisms (*Geotrichum candidum*, *Lactococcus lactis subsp. cremoris* and *Lactobacillus delbrueckii subsp. Bulgaricus*; Clair *et al.*, 2012a). All formulations were highly cytotoxic in all cases, of 10–20 ppm by contrast to glyphosate alone, which was not that toxic in comparison. Roundup causes dose-dependent total cell death within 1 h through an inhibition of the mitochondrial succinate dehydrogenase activity and mostly membrane damages, measured by the leakage of cytosolic adenylate kinase.

Glyphosate is a herbicide supposed to be specific to plant metabolism. Its adjuvants are generally considered as inert diluents. We always observed a greater toxicity of Roundup in comparison to glyphosate alone, and thus side-effects of all its ingredients have been claimed (Benachour and Séralini, 2009). We studied potential active principles for toxicity on human cells for 9 glyphosate-based formulations. The 3 less-toxic formulations were demonstrated

to contain no ethoxylated adjuvants by mass spectrometry, and are around 10,000 times less toxic on mitochondrial activity than POE-15 alone, the major adjuvant. All the other formulations were toxic proportionally to the dilutions of POE-15 or other ethoxylated adjuvants in the formulations. These can be considered as new active principles for human cell toxicity. This is even pointed out in the reviews sponsored by pesticide manufacturers (Williams *et al.*, 2012). Adjuvant toxicity appears to be a general feature of pesticide toxicology, a feature that we studied extensively for this major model of pesticides (Mesnage *et al.*, 2013), but which is also described for other pesticides (Eddleston *et al.*, 2012). We found that for eight major pesticides (out of a total of nine analyzed), the commercial formulation is up to 1000 times more toxic than the active ingredient assessed for safety by regulators (Mesnage *et al.*, 2014). Generally, indications of this problem concerning the considerable increase of toxicity of the supposed active principles of pesticides in commercial formulations by the addition of adjuvants by manufacturers, has already been suggested (Brausch and Smith, 2007; Krogh *et al.*, 2003; Tsui and Chu, 2003).

This does not exclude cellular endocrine disruptions below the levels of cytotoxicity that may not be due to POE-15 alone (or other ethoxylated adjuvants), but that occur at least due to glyphosate (Richard *et al.*, 2005).

10.4.2 Glyphosate action in non-target species

Interestingly, glyphosate tolerance in GMOs is obtained by an overexpression of a transfected and mutated 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) insensitive to glyphosate inhibition. This is because the wild EPSPS responsible for aromatic amino acid synthesis is normally inhibited by glyphosate, which explains its properties as a weed killer. Glyphosate is structurally almost half of an aromatic cycle. It binds to aromatic-recognizing active sites and this is the way it acts (Boocock and Coggins, 1983). In mammals,

glyphosate also inhibited steroidogenic enzymes which have steroid aromatic binding sites, or steroid aromatic receptors (Gasnier *et al.*, 2009); it may therefore act as an endocrine disruptor (Richard *et al.*, 2005). Its adjuvants may help to enter cell membranes including endoplasmic reticulum where aromatase sits (Figure 10.2).

We have measured androgen to estrogen conversion by aromatase activity and mRNA on placental extracts, and demonstrated that glyphosate interacts with the active site of aromatase (Richard *et al.*, 2005). We also observed a human cell endocrine disruption from 0.2 ppm on the androgen receptor in transfected cells, and then from 2 ppm for both estrogen receptors (Gasnier *et al.*, 2009). In freshly isolated rat testicular Leydig cells, non-cytotoxic concentrations of Roundup and glyphosate induced a testosterone decrease by 35% (Clair *et al.*, 2012b).

These results were obtained *in vitro*; cellular cultures are used instead of animal experimentation when possible (Hartung, 2009). Our studies are generally performed over 24 h and do not anticipate the elimination of xenobiotics, their possible bioaccumulation, or long-term combined effects. The human cellular effects of Roundup indeed increased with time (Benachour *et al.*, 2007) and radio-labelled glyphosate accumulated in cells within 48 h, suggesting a bioaccumulation of low concentrations of glyphosate (Gasnier *et al.*, 2011). Roundup adjuvants may also form adducts and link to DNA, avoiding direct elimination (Peluso *et al.*, 1998).

Results were also observed *in vivo*; Roundup altered the spermatogenesis in rats exposed *in utero* to 50 ppm per day (Dallegrave *et al.*, 2007). In a study performed by Yousef *et al.* in 1995, Roundup-treated rabbits presented a decline in body weight, libido, and sperm quality and count (sperm concentration and volume, semen osmolality and also semen initial fructose). Structural and functional testis and epididymes alterations were found in drakes after a 15-day exposure (Oliveira *et al.*, 2007). In rats, the same team showed changes in the progression of
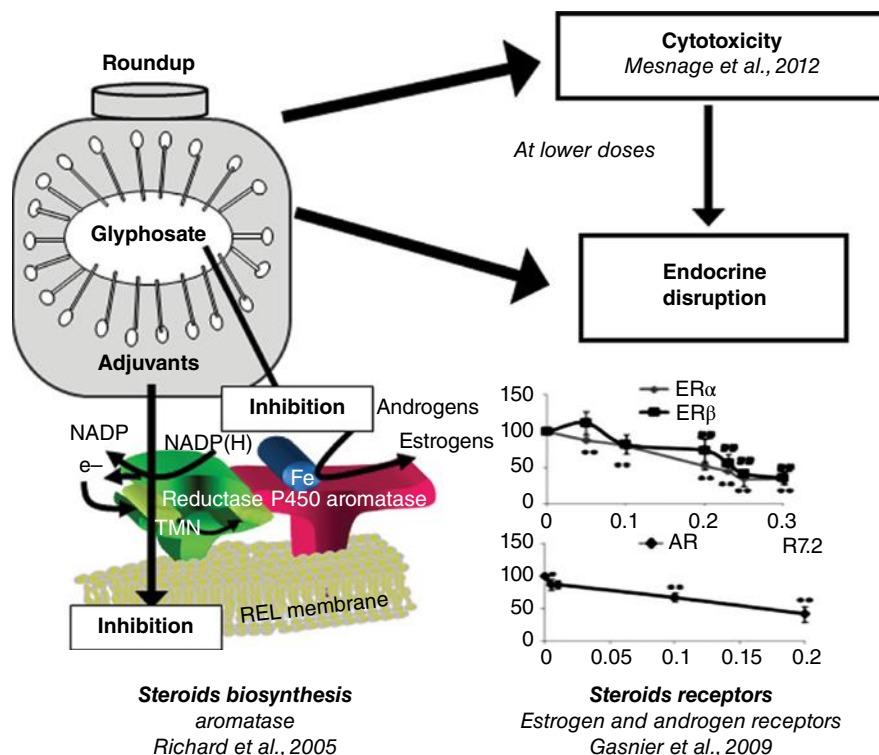
Figure 10.2   Glyphosate and Roundup are cytotoxic and endocrine disruptors at lower levels in human cells. Roundup is highly cytotoxic on human cells because of the non-specific actions of its adjuvants on cell membranes. At lower levels, Roundup is an endocrine disruptor. This is because glyphosate is structurally almost half of an aromatic cycle. It binds to aromatic-recognizing active sites and acts as an endocrine disruptor on aromatase and sex steroids receptors (androgen and estrogens receptors). Its adjuvants may help to enter cell membranes including endoplasmic reticulum, where aromatase is located.

puberty, function and structure of testis in pre-pubertal wistar rats after a 30-day exposure (Romano *et al.*, 2010) as well as on male offspring reproductive development (Romano *et al.*, 2012).

10.4.3   Long-term effects of Roundup or its residues in GMOs

No regulatory authority currently requests mandatory chronic animal feeding studies to be performed on edible GMOs and formulated pesticides. This is why we have recently studied the health effects on rats of a Roundup-tolerant genetically modified maize after a 2-year exposure (from 11% in the diet), cultivated with or without Roundup, and Roundup alone (from 0.1 ppb in water) (Séralini *et al.*, 2012). Our work is the most detailed study involving the life-long consumption of any agricultural GMO and especially on NK603, for which only a 90-day safety test was previously conducted using the same rat strain (Hammond *et al.*, 2004). It is also the first long-term detailed research on any mammal exposed to a highly diluted pesticide in its total formulation with adjuvants (Séralini *et al.*, 2012). We have therefore replicated, extended, and improved the experiments conducted by Hammond and colleagues (Hammond *et al.*, 2004) or other manufacturers for other GMOs in two ways: (1) by measuring outcomes from 3 instead of

2 feeding doses and, more crucially, for a period 8 times longer in duration (2 years versus 90 days) and from (2) 11 blood and urine measures of around 50 parameters (34 organs instead of 17). We have compared the statistical findings that we precisely described (Spiroux de Vendômois *et al.*, 2009) with the results obtained in 90 days by the manufacturer (Hammond *et al.*, 2004) to check if they were biologically relevant or not in the long term.

We biochemically measured 10 rats per sex and group as performed by Monsanto. Even for a study of up to two years, it is indicated in OECD guideline 452 (for chronic toxicity testing) that biochemical effects should be monitored on at least 10 animals per sex per group, even if 20 rats are observed per group/sex.

We emphasized statistically discriminant biochemical effects at the 15th month, when most of animals were still alive (in treated groups 90% males, 94% females, and 100% controls). The significantly discriminant biochemical hepatorenal and sex steroid markers disrupted do correspond to the organic markers linked to the pathologies in a blinded analysis for the pathologists, who in turn linked them to the deaths. In females, all treated groups died 2–3 times more than controls, and more rapidly. This difference was visible in 3 male groups fed on GMOs. All results were hormone and sex dependent, and the pathological profiles were comparable. Females developed large mammary tumors almost always more often than and before controls and the pituitary was the second most disabled organ; the sex hormonal balance was effectively modified by GMO and Roundup treatments. These unexpected effects were found at the lowest dose tested, from 0.1 ppb in water.

Within a week after the release of the publication, two international debates had begun. One debate was scientific, while the other was largely composed of insults and errors. Interestingly, the arguments were mixed in scientific journals. An example was a paper by Arjo *et al.* (2013) published in the journal *Transgenic Research*, a co-author of which was editor-in-chief Paul Christou. The quantity of insults and defamations in this paper is excessive. For instance, Arjo and co-authors suggest that by practicing 'flawed science' we are working against 'progress towards a better quality of life' and are 'actively working to make life worse'. Co-author Christou was previously employed by Monsanto during 1982–1994 and is the inventor of patents used to develop GM Roundup-tolerant maize (Christou, 1996). However, these interests were undisclosed in the published paper. Robinson *et al.* (2013) describe how conflicts of interest are biasing scientific opinion, eroding public trust and scientific integrity, and giving precedence to economic interests over public health.

In a recent development, Elsevier have announced the retraction of the Séralini *et al.* (2012) article (Séralini *et al.*, 2014). After the analysis of all our raw data, the editor-in-chief has stated that there was no fraud, no incorrect data, and no intentional misinterpretation. According to the Committee of Publication Ethics (http://publicationethics.org/files/retraction%20guidelines.pdf), retraction is only justified in the case of error or fraud. In our case, the Editor-in-chief writes that the data are inconclusive because of the rat strain and the number of animals used. These criticisms have however already been answered (Séralini *et al.*, 2013). The decision to retract the paper was reached after the appointment of a former Monsanto employee as Associate Editor for biotechnology in Food and Chemical Toxicology, the journal that published our study. In sharp contrast to our study which provides evidence of toxicity, a study published in the same journal which claims to prove the safety (Hammond *et al.*, 2004) using *the same strain and measuring the same number of rats for serum biochemistry*, is not being subject to the same controversy. According to an editorial in Environmental Health Perspectives (Portier *et al.*, 2014): the decision to retract a published scientific work by an editor, against the desires of the authors, because it is "inconclusive" based on a post hoc analysis represents a dangerous erosion of the under pinnings of the peer-review process, and Elsevier should carefully reconsider this decision. These double standards highlight how economic and political issues are endangering science and public health.

We encourage other research teams to replicate such chronic experiments and to perform, as would be quite logical now, carcinogenesis and developmental studies after our long-term general toxicology study. What is now urgently required is to re-check the burden of experimental proofs of safety for other food/feed GMOs in studies that should be conducted independently of industry. GM NK603 and Roundup cannot be regarded as safe to date.

10.5   Agricultural GMOs producing *Bt* are new insecticidal plants

We recall that the second category of agricultural GMOs synthesizes modified insecticidal toxins out of *Bacillus thuringiensis* (*Bt*) modified transgenes. All natural *Bt* toxins are pore-forming proteins in insect cell membranes (Then, 2010). Since *Bt* toxins have long been used, even in organic farming, their modified counterparts are often compared to them. However, the latter derivatives are truncated, adapted, and contain modified synthetic sequences; consequently their activity is possibly quite different from the natural sequences (Séralini *et al.*, 2011). When a transgenic plant has been modified to produce its own insecticide, most often there is no side-effect-testing, but there is some on bacterial-*Bt*-produced toxins *in vitro*. These can be folded or glycosylated differently in transgenic bacteria and plants, for instance. Moreover, they are used essentially to measure their *in vitro* digestion and stability, and not directly their toxicity on non-target cells. Modified *Bt* toxins are also assumed to be safe through theoretical *in silico* considerations when compared to immunogenicity databanks. The structural and activity comparisons are not scientifically sufficient to predict toxicological effects or safety. For instance, these considerations were unable to predict the toxicity of the pathological prions, hormones and venoms, which are also proteins and are now well known as being far from innocuous. We are back to the same problem as discussed for herbicide tolerant GMOs: the full product is not tested in the manner it is used.

It must be highlighted that the modified *Bt* toxins produced by the GM plants are in soluble forms and thus already biochemically activated, while those produced by *B. thuringiensis* are secreted as inactive precursors (Hilbeck and Schmidt, 2006). The importance of *Bt* toxin activation is clearly shown by membrane actions in human erythrocytes. Their alterations were detected after exposure with solubilized *Bt* toxins, but not in the intact form (Rani and Balaraman, 1996).

In another of our recent studies, we tested for the very first time Cry1Ab produced by MON810 and Cry1Ac *Bt* toxins (10 ppb to 100 ppm) on the human embryonic kidney cell line 293 within 24 h on biomarkers of cell death (Mesnage *et al.*, 2012a). Cry1Ab caused cell death from 100 ppm; this was measured by membrane alterations. This occurred at relatively high concentrations (100 ppm) in comparison to the concentrations produced in GM plants (1-20 ppm; Székács *et al.*, 2010), but this is only in 24 h and does not anticipate chronic effects or combined effects with other factors that have already been demonstrated to play a role on *Bt* toxin toxicity (Then, 2010). Also, this does not anticipate implications of long-term effects.

After *in vivo* consumption by pigs of the transgenic maize MON810 producing Cry1Ab, the results were interesting to interpret. The group of Walsh *et al.* extensively studied MON810 effects. One of their studies revealed the persistence of the modified *Bt* protein during digestion. However, no evidence of Cry1Ab gene translocation or protein penetration in the organs from blood of weaning pigs was observed (Walsh *et al.*, 2011). The authors revealed alterations in the immune responses of the animals, however. In another study by the same group, serum biochemistry differences with controls were found, but they were dismissed because the changes were not accompanied by parallel histological lesions (Buzoianu *et al.*, 2012). However a delay is commonly observed between biochemical markers disruptions and histological lesions. Organic dysfunctions were even revealed at a later stage within chronic periods. We observed differences in biochemical hepatorenal markers after 90-day feeding trials of MON810 in rats in our reanalysis of Monsanto data (Spiroux de Vendômois *et al.*, 2009).

Other short- or mid-term experiments during animal MON810 consumption (short relative to the lifespan of the species concerned), but independent of companies, revealed adverse effects on the immune system in various species including Atlantic salmon (Sagstad *et al.*, 2007), pigs (Walsh *et al.*, 2011), or mice (Finamore *et al.*, 2008). However, the study of the immune system is not mandatory in regulatory tests for biotech firms, and potential effects of GMOs on the immune system cannot be detected by application of the current guidelines.

After all these arguments, it must be highlighted that regulatory and mandatory protocols to obtain commercial-release authorizations ought to be adapted according to state-of-the art scientific knowledge. Protocols must be implemented by the study of intestine histology after GM and/or pesticide consumption, by the measure of digestive and gut wall enzyme activities, and also of the cellular stress and immune responses or allergenicity markers. In general, studies cannot be fully conclusive if the animals are not fed during their whole life while the maize is conceived to be chronically consumed.

10.6   Side-effects of the genetic modification itself

The focus on pesticide residues means that other effects due to the transgene itself or inherent to the technique of genetic engineering are neglected. Genetic engineering is claimed to be an improvement of traditional plant breeding, but in facts consists most often of random and imprecise multiple truncated or abnormal DNA insertions (Uzogara, 2000; Rosati *et al.*, 2008).

Current assessment also relies on the notion of substantial equivalence, meaning that the nutrient composition of the transgenic plant is compared to its isogenic counterpart. This concept has failed to prove GMO food safety and is not supported by recent scientific evidence (Perry *et al.*, 2009). Substantial equivalence has never been precisely characterized, and is more of a commercial and a political judgment rather than a scientific concept (Millstone *et al.*, 1999).

10.6.1   Specific side effects of the transgene expression

In our chronic study similar effects with respect to enhanced tumor incidence and mortality rates were observed in the groups of animals fed with the NK603 transgenic maize without Roundup application. A possible explanation of this finding is the production or alteration of specific compound(s) in the GM feed by the new mutated EPSPS, which could either be directly toxic and/or the cause of an inhibition of pathways that in turn generate chronic toxic effects.

An example of these effects is that the NK603 GM maize used in this study is engineered to over-express a modified version of the *Agrobacterium tumefaciens* EPSPS (Hammond *et al.*, 2004) allowing Roundup tolerance. The modified EPSPS is not inhibited by glyphosate, in contrast to the wild enzyme. This enzyme is known to drive the first step of aromatic amino acid biosynthesis in the plant shikimate pathway. In addition, estrogenic isoflavones and their glycosides are also products of this pathway (Duke *et al.*, 2003). In our study, it was found that they were not disturbed. However, the levels of caffeic and ferulic acids in the GM diets, which are also secondary metabolites of this pathway but are not always measured in regulatory tests, were significantly reduced. This may lower their protective effects against carcinogenesis and even mammalian tumors (Kuenzig *et al.*, 1984; Baskaran *et al.*, 2010). Moreover, these phenolic acids and in particular ferulic acid may modulate estrogen receptors or the estrogenic pathway in mammalian cells (Chang *et al.*, 2006). This does not exclude the action of other unknown metabolites.

This is despite the fact that the variety of GM maize used in this study was judged both by the industry and the regulators as being substantially equivalent to the corresponding non-GM closest isogenic line. As the total chemical composition of the GM maize cannot be measured in detail, the use of substantial equivalence is insufficient to highlight potential unknown toxins and therefore cannot replace long-term animal feeding trials for GMOs.

10.6.2 Insertional mutagenesis or new unexpected/unexplainable metabolism

Hazards could arise from the consumption of the GMO itself. The result of the genetic modification is not a clean insertion and is very imprecise. In fact, the insertion of the transgene can induce wide metabolic changes, for instance, in the MON810 maize where the transgene indirectly introduced 50% changes in osmolytes and branched amino acids (Manetti *et al.*, 2006). This may be due to insertional mutagenesis or a new metabolism. In the case of MON810 maize, 43 proteins were up- or down-regulated with respect to their isogenic lines (Zolla *et al.*, 2008).

The transgene is often altered and other fragments can be introduced elsewhere in the host genome. These changes have been characterized following the insertion of the EPSPS in the Roundup Ready soybean event 40-3-2 (Windels *et al.*, 2001). As a result, new 'fusion genes' from the plant's neighboring DNA sequences of the insertion site can be synthesized (Rosati *et al.*, 2008). The insertion of the transgene may activate, inactivate, under- or overexpress a nearby gene. Very strong promoters indeed are used to overexpress the transgene. The read-through of the new sequences inserted downstream of the EPSPS was processed in four different RNA-variants which might code unknown EPSPS fusion protein (Rang, 2005). Other potentially active compounds such as micro RNAs can be synthesized (Zhang *et al.*, 2012). This is exacerbated by the instability of the transgenic DNA that tends to break, rearrange, delete, or insert elsewhere in the genome, which can be promoted by environmental changes (Matthews *et al.*, 2005), in a transposon-like mechanism of DNA rearrangement.

10.7 Limits and difficulties of interpretations in toxicity tests

All pesticides made from synthesis chemistry are tested on laboratory animals, such as invertebrates or fish, before being marketed. These *in vivo* toxicity tests are supposed to reveal potential adverse effects on mammals, most of the time rats, whose physiology is similar to that of humans; rats also serve as models for other mammals exposed to this feed. An understood side-effect in one mammal should exclude the consumption by others even from an animal ethical point of view. The chronic experiments are based on analyses of blood and organs (after autopsy) and such experiments are conducted for periods ranging from six months to two years, the average lifespan of a rat for the active principle only (at the most). Biotech firms are making different types of tests following very specific standards that they helped develop within the Organization for Economic Co-operation and Development (OECD), which manages international trade. The results, classified as confidential for the public at large as well as for the scientific community, are presented to expert panels in governments, food safety agencies from various countries, or directly to the European Union. This section debates the interpretation of possible side effects.

Doull *et al.* (2007) indicated their general criteria needed to classify the observed significant effects during 90-day toxicological tests on mammals as biologically relevant. The example taken was a GMO, a *Bt* maize called MON863, producing in its cells a new kind of modified insecticide. The authors claim to have applied the same criteria to other products such as pesticides and drugs. Below is the list of criteria used for all commercialized products. They were applied in the reanalysis of the German Health Agency for the renewal of glyphosate approval (German Federal Agency CPFS, 1998). They were strongly criticized because their application has led to the dismissal of glyphosate teratogenic effects (Antoniou, 2012).

1. Many authors make comparisons with historical data of control rats, both within the laboratory and the breeding company from which animals are sourced. However, this clearly enhances control variability and heightens the risk of false negative findings (Yoshimura and Matsumoto, 1994; Cuffe,

2011). It is now established that this concept should be used with caution. There are several reasons for this. Control diets for rats are contaminated with pesticides (Hayes, 2004) or chemicals leaching from cages (Howdeshell *et al.*, 2003); this artificially enhances background effects. The rat suppliers even recognize that their historical data come from rats potentially fed on GMOs (G.E. Séralini, personal communication, 2012). Last but not least, the occurrence of some spontaneous neoplasms in historical controls data is not stable over time and subject to positive or negative time trends (Tennekes *et al.*, 2004). Statistical comparisons therefore cannot be considered as relevant when they are performed between different experimental conditions such as in historical norms. They should focus on controls of the same experiment.

2. The effects have to be plausible. The authors reserve the right not to consider an outcome if they find it unconvincing. This was the case when some doses used in glyphosate precommercial testing that reported adverse effects were considered as too low to elicit a relevant effect. This is not a scientific method.

3. A major gap in some toxicological assessments is the lack of measurements investigating endocrine-disrupting effects (Birnbaum, 2012). The central dogma in toxicology is that effects vary linearly with dose. This is true for standard poison intoxication. However, toxins with endocrine-disruptive properties can give response curves that are U-, inverted-U-, or J-shaped, as frequently observed in the case of exposures to environmental pollutants (Vandenberg *et al.*, 2012). These effects were acknowledged more than a decade ago, and have been extensively reviewed by an expert panel upon the request of EPA confirming non-monotonic dose/effect relationships of endocrine disruptors (Kaiser, 2000). Low-dose effects cannot be invalidated because of the lack of high-dose effects (Myers *et al.*, 2009b). Even if food safety agencies today acknowledge that endocrine-disrupting effects at low doses do occur, they do not change their opinions on commercialized endocrine disruptors such as bisphenol A because they only performed a so-called 'validated' study (Fagin, 2012), meaning studies following international guidelines such as those of OECD. Advice to industrial chemists on how to screen chemicals for endocrine-related effects have now been published (Schug *et al.*, 2013).

4. The occurrence of similar effects in both sexes is an important criterion of toxicity for Doull *et al.*, but is not for us. Sex-dependent differences in chronic diseases resulting from chemical intoxication are well established (Lu *et al.*, 1991; Sissung *et al.*, 2006). This is a major mistake because many organs are sex differentiated; moreover sex-specific chronic diseases are a well-known feature (Kobliakov *et al.*, 1991). We have developed this topic in a review including experimental evidence (Séralini *et al.*, 2009).

5. Chemical, pesticide, drug, or GMOs companies often assumed that biochemical parameters disturbed could be rejected if no association with lesions in histopathology was evidenced. In short-term and mid-term studies, metabolic changes often precede histological changes which may only be detectable in long-term studies.

6. The effects must be always reproducible. This is a very important criteria, but one which must be used cautiously. Toxicities cannot be simply dismissed because they were not consistent between various experiments from different teams to conclude on safety. By contrast, this should be a reason to bring more effort into replicating the experiments and into finding the reason(s) for the lack of reproducibility. In a review of 121 replicate rodent carcinogenicity assays from both sources, only 57% of concordance was found between two classifications (Gottmann *et al.*, 2001). Rodent carcinogenesis bioassays are therefore not easily reproduced and the final decisions for risk assessment have to come from converging evidence at different levels

(*in vitro* assays, structure-activity relationship models, in-cell studies, animal lab studies, farm and wildlife observations, medical reports of workers in the factories where the potential toxic compounds are produced, socioeconomic considerations, etc.). Most of the time, all these data exist but are disregarded by agencies. Data provided by companies with OECD norms are often the main, if not the only, data considered.

7. The assessment of the quality of a toxicological test by agencies often refers to the Good Laboratory Practices (GLP) guide. Generally, it is standard practice that a regulatory agency does not take into account research studies, because they are not conducted under GLP conditions (Myers *et al.*, 2009a). By its very nature, a research protocol is rarely compatible with GLP agreements. A GLP agreement is a good tool to normalize regulatory assessment, but research studies need a greater degree of freedom in test protocols, models, etc. Experimental evidence of the hormonal disturbance induced by bisphenol A have been rejected because they were not conducted in a manner consistent with the GPL.

8. Statistical thresholds are perceived as an absolute truth. In our opinion, statistics do not tell the truth, but may help us in our understanding of experimental outcomes. Biological interpretations and the crossing of methodologies are key (Cooper and Kavlock, 1997).

10.8 The relevance of *in vivo* findings and length of the nutritional tests

10.8.1 Insufficiencies of *in vitro* tests

The usual manner in which agricultural GMOs and formulated pesticides such as Roundup are commercialized with short-term or no *in vivo* testing, as well as keeping raw data hidden for reasons of commercial confidentiality, is certainly not scientifically rigorous. Moreover, the first focus of regulatory assessments of agricultural GMOs should be the toxicity of pesticide residues. Current practice overlooks the fact that all GM crops are genetically modified to contain pesticide residues. These pesticide residues are new elements in our diet, both in type and quantity. The proteins usually compared (modified *Bt* toxins and wild toxins) are not identical, and the tests on human cells of *Bt* proteins are not performed or requested by authorities. Their stability has been assessed *in vitro*, and GM insecticidal toxins are never fully digested *in vivo* (Paul *et al.*, 2010). If some consumers suffer from stomach problems or ulcers, the new toxins will possibly act differently; digestion in children could also be affected. However, these GMOs could be eaten anywhere and all proteins would never be fully decomposed in amino acids by the digestive tract.

We were the only researchers to test modified *Bt* toxins produced in GMOs on human cells (Mesnage *et al.*, 2012a), as regulatory authorizations depend on more theoretical assessments of safety. We also found evidence of toxicity of adjuvants in Roundup-type pesticides, that is to say glyphosate-based herbicides. Adjuvants are routinely assumed to be inert and are not tested for long-term toxicity as part of the regulatory process (Mesnage *et al.*, 2013). In future, these practices may even be extended to pesticides or chemicals in general. This should not be the case within the framework of a rigorous health or environmental risk assessment.

An increasing number of scientists claim that *in vitro* testing and the use of computational models are the future of toxicology, therefore avoiding the use of animal models, with a view to providing a fast answer to the challenge of testing the 10,000 to 30,000 chemicals currently marketed for which the hazard data is insufficient for assessing potential health risks (Betton *et al.*, 1994). The use of physiology-based pharmacokinetic (PBPK) models is advocated to estimate the chemical bioavailability for tissues in order to assess the relevance of *in vitro*

toxicities. Applied to the prediction of *in vivo* embryotoxic effect levels, it cannot fully replace animal studies and sometimes underestimates embryotoxic potential because embryonic stem cell tests do not perfectly reproduce embryotoxicity (Verwei *et al.*, 2006).

The adult human is composed of about 400 cell types (Vickaryous and Hall, 2006), and we are far from fully understanding their responses to chemical exposures and the systemic consequences for a whole organism. In our opinion, these methods may be a promising tool for the search of therapeutic candidate molecules during the research and development approach; they cannot fully replace animal studies for risk assessment, however.

10.8.2   Limitations of 90-day-long tests

The framework for GM food toxicity testing is not precisely defined and there is a huge gap to fill. Some scientists consider that it is not possible to test the long-term toxicity of a GMO. In chemical testing, short- and mid-term tests provide information on acute and sub-chronic toxicities, often the Lethal Dose 50, used to set up the protocol for longer tests. This is not possible for GMOs which are food and feed. However, that does not prevent at all the comparison between the GM feed and its closest isogenic counterpart in the long term.

The acute toxicity approach (less than a month of investigations on rodents with high doses) may yield results which are more proportional to the dose, as it might correspond to a rapid poisoning of the animals (generally with force-fed experiments). However, for many pesticide studies in the scientific literature, some long-term side effects of pesticides at environmental doses are described, but they are not visible in short-term experiments (Hernandez *et al.*, 2008). Sometimes, a 90-day feeding trial is performed. A meta-analysis of the studies performed by the US National Toxicology Program showed that 30% of the toxic effects were neither seen nor predicted during 3-month-long subchronic tests (Betton *et al.*, 1994). They therefore appear insufficient to ensure food safety.

The physiological interpretations of 90-day-based effects are indeed somewhat limited. It is obvious that the 90-day-long trials on mature animals performed today cannot scientifically replace the sensitivity of developmental tests on neonates. A good example is the gene imprinting by drugs that will be revealed only at maturity; this is an important subject of current research, and many findings have been reported for some chemicals such as bisphenol A (Braun *et al.*, 2009; Braniste *et al.*, 2010).

Trans-generational effects occur after epigenetic imprinting by a pesticide (Anway *et al.*, 2005). These effects cannot be detected by standard 90-day feeding trials; instead, they will be visible after many decades by epidemiology in humans if there are any, as illustrated in the case of diethylstilbestrol, which induced female genital cancers among other problems in the second generation (Wise *et al.*, 2005). The F3 multigenerational study is too rarely performed in regulatory tests. This is why, because of the number of parameters disrupted in adult mammals within 90 days in GMO feeding trials performed by the petitioner, it was necessary to independently reanalyze the raw data obtained by Court order afterwards (Séralini *et al.*, 2007; Spiroux de Vendomois *et al.*, 2009). These studies demonstrated possible signs of hepatorenal toxicity that were confirmed after long-term in vivo testing of the same GMO given at similar levels to the same rat strain, with a comparable number of animals (Séralini *et al.*, 2012). Additional tests including long-term periods should be systematically performed to protect the health of billions of people that could directly or indirectly consume these transformed products.

10.8.3   The need for additional tests including long-term tests

In order to take the debate forward, more data are needed. First, our long-term assessment (the only one of this kind) should be repeated with more animals, even if ten animals per sex per group (as we used in our experiment) allows

powerful statistical analysis of biochemical data according to OECD protocols 452 and 453. Some agencies recently admitted, in contrast to their previously published opinion (EFSA, 2008), that there was a need for long-term studies on GMOs (EU Food Policy, 2012).

It has also been acknowledged that no other long-term study has examined the effects of regular consumption of a pesticide on blood parameters. Even data on the short-term effects of Roundup consumption on blood parameters are lacking (CRIIGEN, 2013). Concerning the regulatory assessment of glyphosate, the presumed active ingredient of Roundup, it appears that several agencies accepted the product as safe despite the fact that they were not in possession of the raw data, which remains commercially confidential (Antoniou, 2012).

The outcome of the debate relies on the scientific community having access to the raw data that allowed the commercialization of Roundup and NK603. When this is made public, our raw data (Séralini *et al.*, 2012), having already been given to a notary, will be published on a website making comparisons possible. The Monsanto toxicological data on NK603 maize recently made public by EFSA (January 2013) is not in a statistically usable format, and an agreement with Monsanto is requested. Moreover, the data examined for Roundup authorizations are clearly abnormally lacking. Indeed, data with implications for public health are not related to manufacturing patents and should not be kept confidential. Finally, we would like to suggest a system where companies fund independent research on their products, commissioned by food safety or research agencies and provided to the scientific community online, with long-term testing for all products to which we are likely to be exposed in the long term.

10.8.4   Unraveling the effects of mixtures

In toxicology, unraveling the effects of chemical mixtures is a huge challenge. As a matter of fact, the first mixtures to be scientifically studied should be the formulations of the pesticides containing adjuvants with an active ingredient, which are always used in that form. These formulations are designed to stabilize and enhance the cell penetration of the so-called active ingredients. However, pesticides are administrated as single molecules in *in vivo* chronic tests. Such chronic tests are used to calculate acceptable daily intakes (ADI) which are considered as robust and regulatory objective values, even to predict other combined effects with different compounds such as the estimation of the Hazard Index (HI). This is a major conceptual gap. Indeed, the fact that an ingredient of a mixture (glyphosate in the formulation) is active in plants does not mean *a priori* that this ingredient is the most toxic of the mixture to non-target species, or more toxic by itself than the mixture. However, there is an unexpressed, widely believed hypothesis that the active principle against plant metabolism (glyphosate) is the most toxic compound in a formulation to non-target species. The differential effects between the major formulated herbicides of the world and their active principle glyphosate certainly invalidate this hypothesis.

We have even highlighted ethoxylated adjuvants as new active principles for human cell toxicity, definitively invalidating the use of glyphosate alone as the only active principle in chronic tests. ADI is therefore miscalculated and, to fit with reality, its definition has to be improved by up-to-date peer-reviewed knowledge. This was requested by the CE 1107/2009 regulation. A working group of the European Commission indicated that ADI should be recalculated anyway to offer a proper level of protection (SCCS, 2011), in particular because of co-exposures to chemicals. This is in accordance with our conclusions, in particular for combinations of formulated pesticides. However, any exposure to a single formulated pesticide is at first to be considered as a co-exposure to an active principle and adjuvants. This should be assessed as a priority; otherwise, the theoretical risk assessment of several active ingredients together would be nonsense if these have been underestimated by neglecting adjuvants. The regulatory two-year chronic tests on mammals should be systematically performed with the formulated pesticide to calculate a more realistic ADI and thus to better estimate health risks.

10.9 Conclusions and future outlook

In general, little attention is brought to formulated pesticide effects although this is the keystone of GMO agricultural management. Lessons have to be learnt from early warnings. We are never exposed to single compounds and this is even truer when stacked traits are designed to accumulate residues of different pesticides. Their combined actions have to be assessed. We recognize that all the combinations cannot be tested, but the relevant combinations such as the combined effects of pesticide residues at the level where they co-occur in GM plants should be tested to ensure a proper risk assessment. We call for a public, independent, transparent, and multidisciplinary assessment of GM food and pesticide formulations. Social and economic issues must be considered in light of the fact that over 400 scientists and experts challenged the contribution of GMO-based farming to food security (McIntyre, 2008). As molecular biologists, we are in favor of genetic engineering used as a research tool in closed laboratories, but we are more concerned about public health than economical interests.

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11

What Have We Learnt from the Melamine-tainted Milk Incidents in China?

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Summary

Melamine is a chemical compound which finds its use in industries for the production of plastics, dishware, commercial filters, and others. However, melamine caught the attention of the world due to the economical motivations of adulteration in infant formula during 2008 in China. This chapter describes the melamine adulteration events and discusses: melamine and its food safety incidents related to feed and infant formula adulteration; development of the detection methods of melamine and its analogues; the clinical characteristics and the imaging features of the melamine-induced nephrolithiasis; the diet exposure evaluation of melamine; and what we have learnt from the food safety incident.

11.1 Introduction

Since the spring of 2008, pediatricians in Gansu, Hebei, Beijing, and other provinces noticed an increase of urinary tract stones in infants and young children. According to the report by the Ministry of Health (MOH PR China, 2008), 294,000 infants and young children in China were diagnosed to have a urinary tract stone. The cause of this incident was identified as melamine which was illegally added to milk as an adulterant to give the appearance of increased protein contents. This economically motivated incident resulted in a food safety emergency. A risk assessment of food safety was carried out in China and later by WHO. Scientists from different nations

and non-governmental bodies dedicated their work on the detection method, toxicology and health effects, epidemiology, exposure and risk assessment of melamine and its analogs in milk powder and related products (Wu and Zhang, 2013).

11.2   Melamine and its analogs

Melamine ($C_3H_6N_6$, CAS 108-78-1), 2,4,6-triamino-1,3,5-triazine (Figure 11.1), is a commercially synthesized chemical from urea, which is used in a wide variety of applications including plastics, adhesives, laminates, paints, permanent-press fabrics, flame retardants, textile finishes, tarnish inhibitors, paper coatings, and fertilizer mixtures. The worldwide production of melamine was estimated to be around 1.2 million tons in 2007 (Bizzari and Yokose, 2008). The primary usage of melamine is in the synthesis of melamine formaldehyde resins for the manufacture of laminates, plastics, coatings (including can coatings), commercial filters, and dishware/kitchenware.

The large industrial use can result in the presence of melamine in the environment, which may result in trace levels in the food chain through different ways such as migration from food contact materials or kitchenware. The degradation of other industrially used compounds such as trichloromelamine (used in sanitizing solutions for food-processing equipment and food-contact articles) and triazine-based pesticides/herbicides (e.g. cyromazine) also contributes to the presence of melamine in the food chain.

Melamine may also enter the food chain indirectly as a result of carryover from animal feeds into products of animal origin (e.g. milk, eggs, meat, and fish) that have been treated with products containing melamine (USFDA, 2007; Andersen *et al.*, 2008; Cruywagen *et al.*, 2009; Karbiwnyk *et al.*, 2009; WHO, 2009). Animal feeds can contain melamine as a result of its presence in the environment and from the approved direct addition of precursor compounds such as cyromazine or biuret. It is reported that melamine has been added to binding agents for pellet feeds (shrimp and fish feed) in the USA to improve the binding properties of the pellets (JAVMA, 2007). The inappropriate use of cyromazine or biuret as a ruminant feed additive in animal feed or use of animal feed containing these additives in species for which it is not intended may also lead to melamine contamination in food. Trace amounts of melamine may be found in food as a result of all of the above permitted uses or misuses.

Cyanuric acid ($C_3H_3N_3O_3$, CAS 108-80-5) is also an important industrial product, and is widely used as an ingredient in the production of scouring powders, household bleaches, industrial cleansers, and automatic dishwasher compounds (Kirk, 1993). Cyanuric acid is a stabilizer in swimming pool water to prevent the destruction of chlorine caused by evaporation and sunlight (Kowalsky, 1992). Ammlide ($C_3H_4N_4O_2$, CAS 645-93-2,), ammline ($C_3H_5N_5O$, CAS 645-92-1), and cyanuric acid are byproducts of the manufacturing of melamine. Ammelide, ammeline, and cyanuric acid are also microbial metabolites of melamine if the melamine is not completely metabolized to ammonia and carbon dioxide (Jutzik *et al.*, 1982; Shelton *et al.*, 1997).
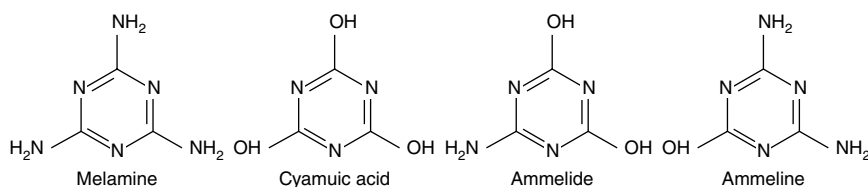
Figure 11.1   Structures of melamine and related compounds

11.3 Melamine incidents

11.3.1 Melamine-contaminated pet food

The first incidence of melamine adulteration with economical motivation was reported in 1982, when 56% of fishmeal samples were adulterated with melamine at 0.25–2.5% (Cattaneo and Cantoni, 1982). Although this was not the first melamine adulteration for the fraud of food or feed, the adulteration of pet food with melamine led to the deaths of hundreds of cats and dogs in the USA in the spring of 2007. Due to the sudden melamine contamination issue, the Food and Drug Administration (FDA) initiated a wide investigation on the etiology of the pathology. This also triggered the testing of other animal feed for melamine, and low levels of melamine were found in feed for fish, poultry, and pigs. An investigation found that melamine and related triazine compounds were present in pet food (Nestle and Nesheim, 2007; Kim *et aln*, 2008). Further investigation by the FDA determined that wheat flour, presented as wheat gluten and rice protein imported from China as pet food ingredients and subsequently incorporated into pet food manufactured in North America, was contaminated with melamine and its analogs (cyanuric acid, ammeline, and ammelide; (Brown *et al.*, 2007; Burns, 2007; Ehling *et al.*, 2007; JAVMA, 2007). It is believed that melamine was added to the wheat flour as feed fraud in order to increase the flour's apparent protein content, allowing the flour to pass for a higher-protein-containing ingredient such as wheat gluten (Brown *et al.*, 2007). Cyanuric acid, ammeline, and ammelide are considered contaminants of melamine and may be generated during the production of melamine or as degradation products (AJVR, 2007; Burns, 2007; Puschner *et al.*, 2007; Cianciolo *et al.*, 2008). Melamine has been added to binding agents for pellet feeds (shrimp and fish feed) in the USA to improve the binding properties of the pellets (JAVMA, 2007). After the pet food incidence, some countries have established regulations to ban the use of melamine in animal feed. These melamine-contaminated products were recalled after the pet food incidence, and it is reported that the use of melamine as a binding agent have been discontinued (JAVMA, 2007).

11.3.2 Infant formula

In 2008, the majority of Chinese babies who were fed with infant formula from cow's milk produced by a Chinese dairy products company (Sanlu Group, Shijiazhuang, China), suffered from nephrolithiasis. Later a chemical raw material (melamine) was detected in the milk powder. Although most patients had no symptoms and signs, acute renal failure occurred in a small proportion of patients. More than 50,000 infants were hospitalized, and six deaths were confirmed (Chen, 2009). This event caused worldwide concern regarding the safety of dairy products (Wu and Zhang, 2013).

11.4 Epidemiological studies

The occurrence of melamine and its analogs in tainted infant formula were comprehensively investigated in order to identify the etiologic factors for the urinary stones epidemic in infants and young children in China in 2008. High prevalence and concentrations of melamine were detected in 87 out of 111 Sanlu infant formula samples with a range from $118\,mg\,kg^{-1}$ to a surprising $4700\,mg\,kg^{-1}$, but with neglected concentrations of cyanuric acid, ammeline, and ammelide (Wu *et al.*, 2009). Further investigation conducted by China State General Administration of Quality Supervision, Inspection and Quarantine (AQSIQ) found that 22 of 109 brands of commercially available formula powder had detectable levels of melamine. The melamine levels in samples from the Sanlu Group reached an astounding $2563\,mg\,kg^{-1}$, while levels ranged from $0.09\,mg\,kg^{-1}$ to $619\,mg\,kg^{-1}$ in formula powder samples from other companies (AQSIQ, 2008). The reasons for the concentrative outbreak of nephrolithiasis and acute kidney injury among children in China were evidentially demonstrated. Epidemiologic studies were necessary to further characterize

this disease and to assess its potential long-term influence (Bhalla *et al.*, 2009).

To cope with the epidemic of melamine-related renal failure in infants, the Chinese government started a program of large-scale screening for urinary stones via free ultrasonographic examinations. Most infants with melamine-related stones were asymptomatic and demonstrated preserved renal function (Lau *et al.*, 2009; Sun *et al.*, 2010). Urinalysis in these infant populations showed proteinuria in about one-third of cases and microscopic hematuria in a small number of cases (Guan *et al.*, 2009). The occurrence of melamine-related urolithiasis was related to both the concentration of melamine in ingested milk products and the duration of ingestion. Cyanuric acid was not detected in all of the cases. Compared to term infants, pre-term infants had a greater risk of developing melamine-related calculi (Lau *et al.*, 2009). Another clinical study on 2085 children from the Gansu Province in China identified stones in 348 cases (17%); males had a higher incidence of stone formation than females (216 cases were males while 132 cases were females). Case studies in China also showed that infant age may have been a factor in the rate of stone development, as a higher proportion of stone occurrence was observed in neonates and infants younger than 6 months of age and gradually decreased in infants up to >30 months (WHO, 2008b). Elsewhere, it was suggested that age, sex, and the use of formula alone or in combination with breast milk were not significantly associated with the presence of stones; however, pre-term birth was exceptional (Guan *et al.*, 2009). As well as clinical examinations, many research investigations have also been published relevant to epidemiology, diagnosis, clinical symptoms, and treatment of melamine.

The melamine-tainted infant formula incident occurred almost 5 years ago. More than half of the urinary stone cases recovered after a short hospital stay; however many infants and young children still had residual stone symptoms at the time of discharge. Members of the public are rightly concerned about the epidemic and long-term follow-up. A prospective observational

cohort (8335 children ≤6 years old) study showed that the levels of abnormal urinary microprotein excretion (microalbumin/creatinine and immunoglobulin G/creatinine) were significantly higher in children with persistent stones than in those who passed their stones, indicating that early passage of a stone may reduce the renal injury induced by melamine-contaminated milk-powder-associated urolithiasis (Gao *et al.*, 2011).

After a period of 1–2 years, several cohort studies in infants and young children from Beijing, surrounding provinces and Hong Kong regions in China demonstrated that the damage to the kidney was temporary with no persistent negative outcomes (Kong *et al.*, 2011; Shen *et al.*, 2011). Conservative treatment including intravenous or oral hydration to increase urine output and urine alkalinization shows a high effectiveness in cases with residual melamine-related urinary stones (Wen *et al.*, 2011). Long-term follow-up cohort studies should be continuously conducted to further investigate the epidemic and chronic hazard of melamine-induced nephrotoxicity.

11.4.1   Emergency exposure assessment in China and WHO

Following the infant formula incident, a dietary exposure assessment was carried out by the Chinese Center for Disease Control and Prevention (China CDC) to investigate the occurrence and concentrations of melamine and its analogs in tainted infant formula and to identify the etiologic factors. A high prevalence and concentrations of melamine were found in Sanlu infant formula samples, with negligible concentrations of cyanuric acid, ammeline, and ammelide. Melamine was detected in 87 out of 111 Sanlu infant formula samples with a range of 118–4700 mg kg^{-1}. The present results showed the Sanlu infant formula samples had an unacceptable level of melamine (Wu *et al.*, 2009). Furthermore, the high level of melamine was found in the raw milk and raw adulterated criminal materials obtained from the milk collection station. The dietary exposure on the basis of the consumption of melamine-adulterated infant formula in China at the median

levels of melamine reported in the most-contaminated brand was estimated to be in the range of 8.6–23.4 mg kg^{-1} body weight per day, which is about 40–120 times the tolerable daily intake (TDI) of 0.2 mg kg^{-1} body weight set by WHO (2008c, 2008d). The results provided strong evidence for melamine as the etiological factor for the urinary stones epidemic in infants and young children in China in 2008.

Upon the sudden infant formula incidence in China, WHO in collaboration with FAO organized a meeting to review toxicological aspects of melamine and cyanuric acid in Ottawa Canada during 1–4 December of 2008. Those attending summarized and agreed the infant formula survey data. In the health risk assessment, Health Canada tested 80 different infant formula products available on the Canadian retail market (Health Canada, 2008). Melamine concentrations were in the range of 4–346 µg kg^{-1} in formula as purchased, with 75% of samples containing detectable levels. The equivalent concentrations on an 'as received' basis were in the range 0.5–69 µg kg^{-1}. Dietary exposure assessments were performed for different infant age groups using published food consumption data for each group (Institut National de Santé Publique du Québec 2001) or for premature infants using a Health Canada estimate of consumption (Health Canada, 2008). Other national survey data on infant formula from the USA (USFDA, 2008), New Zealand (J. Reeve, personal communication 2008), and Australia (J. Baines, personal communication 2008) were available; results were consistent with the findings of the Health Canada survey. Dietary exposure assessments were undertaken for different infant age groups using published food consumption data for each group (Institut National de Santé Publique du Québec, 2001) or for premature infants using a Health Canada estimate of consumption (Health Canada, 2008). The dietary exposure assessment used mean concentration and consumption data, and 'non-detects' was set to one-half the detection limit of 4 µg kg^{-1}. Estimated dietary exposures to melamine were 0.54–1.6 µg kg^{-1} body weight per day.

11.4.2 Initial and later risk management responses of Chinese government

On identifying the cause, the Chinese government implemented a series of measures to control this crisis. With the joint efforts of several ministries, the melamine adulteration crisis was under control within one week. The Chinese government promulgated an interim control limit for melamine at 1 mg kg^{-1} for infant formula and at 2.5 mg kg^{-1} for other milk products on 7 October 2008, and this interim limit became official on 6 April 2011. Many inspections regarding the safety of milk and milk powder products were conducted by food-safety-related ministries including the Ministry of Health (MOH), the Ministry of Agriculture (MOA), AQSIQ, etc. The inspection data showed that all dairy products, including infant formula, have been in compliance with the control limits of melamine since September 2008. Based on the scientific findings, the Chinese government took a series of risk management measures. Raw milk and infant formula production were subjected to intensive regulatory control and inspection, including: inspectors were stationed at every major dairy product manufacturer; each batch of raw milk was tested for melamine; and interim control limits for melamine were set up for infant formula and other dairy products (1 mg kg^{-1} for infant formula and 2.5 mg kg^{-1} for liquid milk and adult milk powder; USFDA, 2008; Wu *et al.*, 2009).

In addition, guidelines for the diagnosis of urinary tract stones in infants and young children were quickly developed. Free medical check-up was provided by the hospitals for any children who had consumed the milk powder under suspicion; within 3 months, more than 20 million children had been examined by ultrasound B. Free hospitalization and treatment were also provided for affected children. Although the prognosis of the identified cases is expected to be good, in order to ensure the health of the patients, they are followed up every six months.

This case of melamine adulteration of milk fraud led to criminal convictions and subsequent

compensation to the affected children before the Chinese New Year in January 2009. Proper risk communication among all the stakeholders relevant to food safety has proved to be very important in crisis management. The State Council immediately released a list of all 22 dairy companies whose infant formula products had been found tainted with melamine. Continuous information release during the crisis from the Government on the toxicological characteristics of melamine, testing results of melamine in dairy products, control actions taken by the government agencies, interim control limits for melamine in infant formula and other dairy products, and policies on free check-up and treatment of patients, made the consumers aware of the details of the crisis and reduced unnecessary anxiety among consumers. Frequent press conferences organized by the State Council and MOH since September 2008 were considered a successful method of risk communication (Chen, 2009).

Nearly 5000 dairy standards have been reviewed by the Chinese government since 2008, with the aim of eliminating repetition, contradiction, and crossing of current dairy standards in order to improve food quality standards. A unified set of the national dairy safety standards was revised and issued by China MOH in 2010, which included more than 160 national dairy standards (66 new dairy standards, 2 standards on good manufacture practice, and 49 standards on detection methods). In addition to the new set of the national dairy safety standards, MOH also issued a list of non-food ingredients which would be classified as illegal food fraud and abuse of food additives; the list includes melamine. On 6 April 2011, MOH issued and immediately enforced another interim limit of melamine which covers both baby food and all other food.

Much has been learnt from the melamine incident, including the proper implementation of risk analysis and the fact that interaction among risk assessment, risk management and risk communication are critical and necessary.

11.4.3 Development of detection of melamine and its analogs in food

The pet-feed fraud with melamine in the spring of 2007 in USA and the adulteration of milk and milk products with melamine in China in 2008 have promoted analytical method validations and sample investigations of melamine and its analogs worldwide. Various government administrations, university institutes, and inspection agencies focused on the determination of melamine and related analogs (such as ammelide, cyanuric acid, and ammeline) in milk, milk ingredients, component products containing milk-derived ingredients, and even animal foods using different methods. Chinese dairy products and other mixed foods containing dairy ingredients manufactured or sourced from China caused the greatest concern. The measurement of background levels of melamine in these types of products for imported and domestically produced items was conducted. However, in many cases, the presence of cyanuric acid, ammeline, and ammelide was not tested for.

11.5 Screening methods

11.5.1 Enzyme-linked immunosorbent assay

Commercial enzyme-linked immunosorbent assay (ELISA) kits were developed by various manufacturers for the rapid screening and semi-quantitative determination of melamine following the crisis (Garber, 2008). In China, some commercial ELISA kits and cards were promptly developed by some manufacturers, such as Hexagonal Body Technology Development Corporation and the Beijing Vader Weikang Biological Technology Corporation. All the ELISA kits and cards were widely used for the rapid screening of melamine, offering a solution that satisfied the requirements of high-throughput screening of samples. ELISA technology is sensitive to melamine and ammeline, the main components for the high-throughput analysis. The main weakness of this assay however

was significant cross-reaction. Such interference compounds have a similar structure to melamine, such as ammeline, cyanuric acid, and cyromazine which demonstrated 10%, 0.05% and 300 % cross-reactivity respectively (Lutter *et al.*, 2011), while these related analogs were present in milk samples.

For screening purposes, the immunoassay is advantageous compared to complex instrumental methods because of its high throughput and rapid turnaround time. As well as ELISA, immunogold chromatographic assay (IGCA) and fluorescence polarization immunoassay (FPIA) were also developed for the screening of melamine. IGCA may be an alternative to ELISA as a rapid screening method. The sample pretreatment for IGCA was simple and rapid, and results could be obtained within 3–10 min (Li *et al.*, 2011). FPIA was one of the most extensively used homogeneous techniques, and meets the requirements of a simple, reliable, fast, and cost-effective analysis. The limit of detection (10% inhibition) was 9.3 ng mL^{-1} for determination of melamine in milk and milk powder, and there was no cross-reactivity to other natural structurally related compounds (Wang *et al.*, 2011). Further, fast and screening technology such as an immuno-chromatographic strip test (Li *et al.*, 2011) for detection of melamine in raw milk, milk products and animal feed was also developed.

11.5.2   High-performance liquid chromatography

High-performance liquid chromatography (HPLC) is a commonly used screening method for detecting melamine. HPLC techniques have been applied to the simultaneous detection of melamine, ammeline, ammelide, and cyanuric acid in rice, wheat, and corn flours (Ehling *et al.*, 2007). In addition, some HPLC-UV (ultraviolet) or HPLC-DAD (diode array detector) methods have been validated for the quantification of melamine in infant formula or milk products, eggs, protein powders of plant origin, and pet food. However, HPLC methods could not confirm the target analyte. The UV

spectra of melamine exhibited absorption bands below 250 nm; erroneous quantification could occur if insufficient care was paid to chromatographic conditions or sample preparation was not optimized (Desmarchelier *et al.*, 2009). Several methods were employed to resolve these problems. In order to confirm the retention time, the spectrometric profile of melamine recorded at 240 nm was usually used to confirm the identity of the selected peak. The use of acetonitrile-free extraction solvents (Venkatasami and Sowa, 2010) and ultrasonically assisted extraction method (Zhou *et al.*, 2010) improved the pretreatment procedures and promoted the robustness of HPLC method. Different detectors were used for the quantitation analysis, including DAD systems and fluorescence reactions.

Both ELISA and HPLC were evaluated as reliable methods for semi-quantitative determination of melamine in milk products and also fit for stated limits requirements (1 mg kg^{-1} in infant formula). However, both of the two analytical techniques are limited in terms of specificity. For ELISA, there were cross-reactivity properties towards melamine's analogs or the related triazine pesticide cyromazine, which could result in false positive results. Also, UV and DAD for HPLC separated samples can be regarded as having poor selectivity because many organic compounds absorb in the wavelength range between 200 and 270 nm (Tittlemier *et al.*, 2010). Confirmatory detection is therefore highly recommended after screening.

11.5.3   Capillary electrophoresis

Capillary electrophoresis (CE) is a rapidly growing separation technique in which high-electric-field strengths are used to separate molecules based on differences in charge, size, and hydrophobicity. Compared to other chromatographic methods, CE has some advantages such as high separation efficiency, high speed, and low consumption of solvent and sample. Although two main disadvantages are often referred to (the lack of sensitivity and the low reproducibility), CE has been used as an effective method for the analysis of melamine and related

compounds in milk or milk products. The contents of melamine and related compounds in milk or milk products were within the range 1.32–33.84 µg mL^{-1}. The CE-DAD technique was also used to detect melamine in dairy product, fish feed, and fish (Yan *et al.*, 2009). Nevertheless, many options have been proposed by skilled researchers to overcome the above flaws. Xia *et al.* (2010) optimized a capillary zone electrophoresis (CZE) method via extraction with acetonitrile/water/diethylamine and separation with disodium hydrogen phosphate buffer and UV detection at 214 nm. Himmelsbach *et al.* (2010) also developed a fast and reliable method based on CZE coupled to quadruple time-of-flight (Q-TOF) mass spectrometry for the analysis of methylated melamines. Overall, CE can be regarded as a good alternative to HPLC with the advantage of shorter analysis time and lower cost.

11.6   Confirmatory methods

11.6.1   Gas chromatography mass spectrometry

Gas chromatography mass spectrometry (GC-MS) can be applied for melamine and its analogs quantification and confirmation. Because of the low molecular weight of melamine and its analogs, they should be derivatized into larger molecular weight before analysis. A GC-MS screening method was initially developed by the US FDA, in which melamine was derivatized with trimethylsilyl (TMS) agent in a sample extract prior to analysis. Tzing and Ding (2010) developed a reliable, sensitive and eco-friendly injection-port TMS derivatization and GC-MS/MS method to determine melamine and cyanuric acid in powdered milk samples. Zhu *et al.* (2009b) reported a sensitive instrumental analytical method for the simultaneous determination and confirmation of melamine and cyromazine residue in animal-derived food by using GC-MS, with a limit of detection (LOD) of 10 µg kg^{-1} for muscle tissue and 5 µg kg^{-1} for milk or eggs. In order to further increase the accuracy of the method, an internal standard (IS) was chosen for quantitation during analysis.

2,6-Diamino-4-chloropyrimidine (DACP) was applied as an IS for the quantitative analysis of melamine and its related analogs by GC-MS/MS in milk and milk products with an LOD of 2 µg kg^{-1} (Miao *et al.*, 2009), which was strongly suggested to be used to monitor of melamine adulteration in dairy products during 2008 in China. Perfluorotributylamine (PFTBA) was also considered as an alternative to the use of an IS during GC-MS or GC-MS/MS analysis. In addition, isotope-labeled melamine was also used as an IS for quantitative analysis of melamine in eggs and dairy products (Xu *et al.*, 2009), which demonstrated higher accuracy and precision with recoveries between 93.9% and 102% and relative standard deviation within the range 3.1–8.7%.

Due to its high sensitivity, GC-MS and GC-MS/MS have been widely used for the quantitation of melamine and its analogs in foods. However, tedious sample preparation and clean-up procedures make the methodology impractical and inefficient for analyzing a large number of samples; there is also a high uncertainty of the derivatized efficiency. A simple, effective, and reliable method of detecting melamine and its analogs in food is therefore still needed.

11.6.2   Liquid chromatography mass spectrometry

Liquid chromatography mass spectrometry (LC-MS) and LC-MS/MS are the main methods employed for quantitative determination of melamine due to their molecular specificity and high sensitivity. Milk, milk powder, dairy products, animal-origine food, and biological materials such as serum and urine are usually analyzed by LC-MS/MS method. LC-MS/MS is further used for the tracking and monitoring of melamine metabolites and its biomarkers *in vivo*. Considering the polarity of analyses, hydrophilic interaction liquid chromatography (HILIC) becomes important in the separation of melamine and its analog compounds and interfering materials (Patel-Predd, 2006; Andersen *et al.*, 2008). Related studies used silica-based Venusil-HILIC or zwitterionic-HILIC to

elute well-resolved melamine peaks from various samples, including infant formula. Overall, highest selectivity and reliability is achieved by LC-MS/MS which prevents erroneous quantification, ensuring selectivity of the detection by selected reaction monitoring-based acquisition of melamine mass and quantification on a fragment level.

11.6.3   Matrix-assisted laser desorption/ionization mass spectrometry

Most of the methods described in Sections 11.6.1 and 11.6.2 require extensive sample pre-treatments which are costly and time consuming. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is another effective method for quantitative analysis of melamine and its analogs in food items. The merits of this method are simple sample preparation and short analysis time. It can be used for quantitative analysis of melamine, cyanuric acid, ammelide, and ammeline in pet food, urine, and milk samples. The three commonly used MALDI matrixes, namely, α-cyano-4-hydroxycinnamic acid (CHCA), sinapinic acid (SA), and 2,5-dihydroxybenzoic acid (DHB), are able to desorb/ionize melamine from melamine cyanurate (MC) upon N_2 laser irradiation. CHCA demonstrates the highest detection sensitivity in the positive mode, and DHB and SA in the negative mode (Tang *et al.*, 2009).

In situ analysis is increasingly needed in food analysis. To date, several novel MS-based techniques, including low-temperature plasma (LTP), desorption electrospray ionization (DESI) (Yang *et al.*, 2009), and extractive ESI (Zhu *et al.*, 2009a), have been reported for rapid *in situ* analysis of melamine in complex mixtures, with minimal or no sample pretreatment. New ionization methods by the LTP probe (Tang *et al.*, 2009) and the nanoDESI (Hoogland and Boon, 2009) for the determination of melamine in complex matrices were developed, making the MALDI-MS method more sensitive. Overall, MADLI-MS methods can provide rapid analysis, high sensitivity, and high specificity.

However, the instrument is the most expensive and sophisticated, resulting in its low practicality.

11.6.4   Application of new technologies

Further to LC-MS, GC-MS, and MALDI-MS methods, some other techniques have been applied to the determination of melamine and its analogs in food, feed, or biomaterial samples. These include: colorimetric detection in which non-aggregation-based Au-NPs are used as a probe (Cao *et al.*, 2010); micellar electrokinetic chromatography (Tsai *et al.*, 2009); differential pulse polarography (Yilmaz and Yazar, 2012); electrochemical sensor (Liang *et al.*, 2009) and optical biosensor (Fodey *et al.*, 2011); flow-injection chemi-luminescence determination (Zeng *et al.*, 2011) and chemi-luminescent enzyme immunoassay (Choi *et al.*, 2010); and resonance scattering detection (Jiang *et al.*, 2011). On the other hand, the simplest method of visual detection (Li *et al.*, 2010) has also had a role in the field of melamine determination. Some innovative technologies, such as one-step synthesis of silver/dopamine nanoparticles (Ma *et al.*, 2011), injection-port derivatization (Tzing and Ding, 2010), and polymer monolith microextraction (Liu *et al.*, 2010) have also been used.

11.7   Health effects and toxicology of melamine and its analogs

11.7.1   Health effects

After the melamine events in 2007 (USA) and 2008 (China), the WHO, FDA and other relevant professional institutes launched a research effort into the health effects of melamine and its analogs. Presently, kidney and liver are considered to be the target organ of melamine toxicity effects. Melamine and its analog cyanuric acid are not metabolized and are rapidly eliminated in the urine with a half life in plasma of around 3 h or 1–2 h, respectively (OECD, 1998, 1999). Melamine

or cyanuric acid alone is of low toxicity. Experimental studies have however shown that melamine combined with cyanuric acid leads to severe kidney stones and bladder stones, with subsequent nephritis, cystitis, renal failure, and bladder epithelial cell hyperplasia, as well as liver damage (Melnick *et al.*, 1984; Dobson *et al.*, 2008; Bhalla *et al.*, 2009).

11.7.2   Toxicology

Based on the available reports, melamine and cyanuric acid are not metabolized and rapidly excreted in the urine of mammal animals (OECD, 1998, 1999). The target for melamine or cyanuric acid toxicity is the urinary system in monogastric animals, including human beings (Gao *et al.*, 2011). A persistent effect of melamine in experimental animals is kidney stones and bladder stones (Dalal and Goldfarb, 2011). Stone formation has also been reported following exposure to a certain dose of melamine or cyanuric acid (Gamboa *et al.*, 2012). Carcinogenic effects of melamine are considered to be secondary to irritation caused by stones. Melamine exposure together with cyanuric acid can induce acute melamine–cyanurate crystal nephropathy, subsequently leading to renal failure at much lower doses than with either melamine or cyanuric acid alone (Cianciolo *et al.*, 2008). There are very few data on melamine analogs other than cyanuric acid, even although ammelide and ammeline as analogs of melamine are assumed to be of equal potential health risk. In the absence of data, it was necessary to rely on toxicological studies in laboratory animals or cell lines to characterize the human health risk related to melamine and/or cyanuric acid in food.

11.7.3   Toxicity of melamine

There are no human data on the oral toxicity of melamine. Most studies report the available toxicology data from studies in rats, mice, dogs, pigs, sheep, or other mammal animals (Baynes *et al.*, 2008, 2010; Yoon, 2011). Toxicity can be classified as acute or chronic. The most common toxicity is renal toxicity, which is also the area of most concern to nephrologists.

11.7.3.1   Acute toxicity
The acute toxicity of melamine is low. In the presence of its analog and common co-contaminant, cyanuric acid, toxicity is much higher. Both are excreted via the kidney without metabolism with half-lives estimated to be a few hours. Each may form crystals in urine. When both are present in urine, they can combine as melamine cyanurate complexes. This results in highly insoluble, larger, and more numerous calculi than either of the two chemicals alone. These irritate the urinary tract lining and may lead to obstruction and uremia. Melamine cyanurate crystals have been implicated as the cause of renal toxicity in cats and dogs resulting from contaminated pet food in 2007 (Brown *et al.*, 2007). The signs and symptoms of acute melamine exposure (high dose over several days) and presumably in the presence of a co-crystallizing substance, is related to acute urolithiasis or cystitis (e.g. anorexia, hematuria, abdominal pain, oliguria, uremia; Xie *et al.*, 2010).

11.7.3.2   Chronic toxicity
Most of the sub-chronic and chronic feeding studies in animals have failed to show any renal toxicity. In female rats however, a 13-week feeding study found dose-related calcareous deposits in the proximal tubules. Following a 2-year feeding study, chronic inflammation of the kidney was found (DHHS/NTP, 1983). In rats and dogs, high doses of melamine had diuretic properties but did not produce renal toxicity (Lipschitz and Stokey, 1945).

11.7.3.3   Carcinogenicity
There are no publications regarding the carcinogenicity of melamine in humans. Carcinogenicity in animals was determined from studies in rats and mice. Exposure produces urinary bladder and urethral transitional cell carcinomas in male rats, but only urinary bladder hyperplasia in male mice. Female rats or mice did not have carcinoma, but transitional cell

papillomas were found in female rats (Mast *et al.*, 1983; JMPR, 2006). The occurrence of urinary bladder tumors in male rats correlates well with stone formation and exposure to high dosages (Melnick *et al.*, 1984). A similar dosage-dependent relationship was confirmed in another study using male rats (Ogasawara *et al.*, 1995). The administration of sodium chloride to increase fluid intake and urinary output reduces the prevalence of stone and tumor occurrence. There is no evidence that melamine undergoes biotransformation. Mutagenesis of melamine was not observed in studies of exposure to *Salmonella typhimurium* and *Drosophila melanogaster* (Mast *et al.*, 1982, 1983). The urinary bladder tumors seen in male rats exposed to high dosages of melamine seem to be produced by a non-DNA-reactive mechanism involving epithelial hyperplasia secondary to the presence of melamine-containing bladder stones (DHHS/NTP, 1983). These studies concluded that bladder tumors would not occur in rodents unless exposed to dosages that result in bladder stones. Melamine has been classified as a category III carcinogen by WHO, meaning that melamine is not classifiable as to its carcinogenicity in humans, referring to the fact that the evidence of carcinogenicity is inadequate in humans and inadequate or limited in experimental animals (IARC, 2006).

11.7.3.4   Genotoxicity

Results of a genotoxicity study showed that melamine has no mutagenic function in prokaryotic and eukaryotes both *in vitro* and *in vivo*, and a low dose could not induce malignant cell transformation after long-term exposure (Mast *et al.*, 1982, 1983; WHO, 2008a). However, it has the ability to increase sperm abnormality rate and damage DNA (Zhang *et al.* 2011). Based on these results, the WHO International Agency for Research on Cancer has concluded that there is sufficient evidence in experimental animals for the carcinogenicity of melamine under conditions in which it produces bladder calculi (IARC, 1999). There is inadequate evidence for carcinogenicity in humans.

11.7.4   Toxicity of cyanuric acid

Cyanuric acid has low acute toxicity in mammals, with a rat oral LD50 of 7700 mg kg^{-1} body weight for rats (OECD, 1999). Several subchronic oral toxicity studies have demonstrated that it causes renal tissue damage, including dilatation of the renal tubules, necrosis or hyperplasia of the tubular epithelium, increased basophilic tubules, neutrophilic infiltration, mineralization, and fibrosis. These changes were probably caused by cyanurate crystals in the renal tubules (OECD, 1999). The no-observed-adverse-effect-level (NOAEL) for these effects is 150 mg kg^{-1} day^{-1} (OECD, 1999). In humans, more than 98% of an orally administered dose of cyanuric acid is excreted unchanged in urine within 24 hours (Allen *et al.*, 1982). Sodium cyanurate was tested in several short- and long-term studies in rats and mice. It did not induce any genotoxic, carcinogenic, or teratogenic effects. Observations in rats and mice at high doses were of the occurrence of bladder calculi, epithelial hyperplasia of the bladder, and, during longer-term studies, renal tubular nephrosis (Hammond *et al.*, 1986; Cremonezzi *et al.*, 2004). The NOAEL for sodium cyanurate derived from the 2-year study in rats was 154 mg kg^{-1} body weight per day (WHO, 2004).

11.7.5   Combined toxicity

Evidence from the outbreak of acute renal failure in cats and dogs in 2007 associated with the consumption of contaminated pet food suggests that when melamine and cyanuric acid are co-ingested renal toxicity results (Brown *et al.*, 2007). In the pet food incident, analysis of the food revealed the presence of a number of triazine compounds in the feed including melamine and cyanuric acid. A small study in which cats were fed increasing amounts of melamine and cyanuric acid also reported renal failure and the presence of renal crystals (Brown *et al.*, 2007; Puschner *et al.*, 2007). This was confirmed by Dobson *et al.* (2008) who conducted a rat study that tested ingestion of melamine alone, ammeline or ammelide alone (both analogs of melamine), a mixture of melamine

and cyanuric acid, and a mixture of all four compounds. Neither ammeline nor ammelide alone produced any renal effects, but the mixtures produced significant renal damage and crystals in nephrons (Gamboa *et al.*, 2012). Analysis confirmed the presence of melamine and cyanuric acid in the kidney. Infrared microspectroscopy on individual crystals from rat and cat (from the pet food outbreak) kidneys confirmed that they were melamine-cyanuric acid co-crystals. Melamine cyanurate has very low solubility and it is hypothesized that this leads to the formation of melamine cyanurate crystals in the kidney. It is assumed that melamine and cyanuric acid are absorbed in the GI tract, distributed systemically, and, for reasons that have not yet been fully determined, precipitated in the renal tubules leading to progressive tubular blockage and degeneration (Dobson *et al.*, 2008).

From the best-available evidence in human exposure and animal studies of the toxicity of melamine, the following conclusions can be drawn: high-dosage melamine will result in urinary stones, crystalluria, and acute renal failure in both humans and animals; stone formation is likely enhanced by smaller body size, higher dosage of melamine, and smaller amounts of fluid intake; studies in animals show that males are more affected than females; toxicity of melamine is further aggravated by the presence of other impurities associated with melamine synthesis, particularly cyanuric acid; tubular damage with obstruction from crystals and chronic inflammation of kidney can occur; and toxicity may not be limited to stone formation in animal studies if melamine is present in high dosages or in combination with cyanuric acid (Bhalla *et al.*, 2009; Hau *et al.*, 2009; Skinner *et al.*, 2010).

11.8   Diet exposure assessment from China Total Diet Study

Analytical methods are available for the screening and quantification of melamine and its analogs in food and feed, such as milk, eggs, meat and other diet samples. Until the end of 2009, most published research was on the topic of infant exposure to melamine; data on adult exposure was rare. The average Chinese daily intake of melamine was estimated in the 4th China Total Diet Study (TDS). A TDS enables the estimation and monitoring of dietary exposures to chemical residues, contaminants, and nutrient elements as consumed which involves purchasing at the retail level foods commonly consumed by the population, preparing them as for normal consumption, homogenizing and compositing them, and, finally, analyzing the foods for the chemicals of interest (WHO, 2005). WHO strongly recommends that member countries conduct their own TDSs, as it is one of the most cost-effective means of ensuring that people are not exposed to unsafe levels of toxic chemicals through food. The Global Environment Monitoring System/Food Contamination Monitoring and Assessment Programme (GEMS/Food) by WHO has also encouraged member countries and developing countries to undertake TDSs as a matter of public health significance, while recognizing the its importance to standards development and status evaluation of their food safety.

Beginning in 1990, the China TDS has become an important tool for monitoring dietary exposures to chemicals and their associated risk to public health; such studies have been undertaken five times in China at various intervals. The 4th Chinese TDS was conducted during 2007–2008. The 4th China TDS samples of Hebei province were collected at the beginning of year 2008. The diet samples of the 4th China TDS, including 48 composite animal original diet samples, were determined to evaluate the associated health risk. Most of the 4th China TDS samples of animal origin demonstrated very low levels of melamine. However, a higher concentration of melamine was detected in the composite HeBei milk product sample ($176.35\,\mathrm{mg\,kg^{-1}}$) compared with that in the other TDS samples, as would be expected from the consumption of melamine-contaminated Sanlu infant formula in 2008. The diet exposure assessment results indicated that residents in HeBei province would be exposed to 37.1% of the health-based reference health value of TDI

of 0.2 mg kg^{-1} bw (WHO, 2008d), resulting a lower-degree health risk (Fan Sai, personal communication, 2013). Since the analogs of melamine were also detected during the 4th China TDS, the TDI established by the FDA (0.63 mg kg^{-1} bw per day with the additional 10-fold safety factor) was adopted to calculate the dietary exposure of melamine and its analogs. From the exposure data, the health risks were at a lower level in most of the provinces except for HeBei province. The dietary exposure of melamine and its analogs of HeBei province were 75.290 μg kg^{-1} bw day^{-1}, which is 119.8% of TDI. However, the levels of cyanuric acid, ammeline, and ammelide in composite animal origin 4th China TDS samples of HeBei were no more than 1% of melamine. Most of the ingredients contaminated in the composite animal samples of the 4th China TDS were therefore melamine. On consideration of this, the TDI of 0.2 mg kg^{-1} bw (WHO, 2008d) established by WHO was adopted.

Based on the available reports, a high prevalence and concentration of melamine and its analogs were found in the composite and individual milk products samples of the 4th China TDS from HeBei province, but no melamine or its analogs were detected in the samples of the 3rd China TDS which was conducted in 2000. The diet exposure assessment provides a rational explanation for the affected infants in 2008.

11.9   Who should be responsible for food safety in China?

Safe food is a prerequisite for health, and ensuring food safety is a public health priority in China. According to Dr Margaret Chan, WHO Director-General: 'Governments need to give food safety just as much attention as they devote to the quality and safety of pharmaceutical products. Not everyone needs to take medicine every day but all people need food each and every day.'

Nowadays, food safety is not only one of the most striking and important public health issues worldwide, but it has also become one of the most challenging social issues in China that needs to be addressed, especially since the melamine incidence in 2008.

11.9.1   Food safety is the responsibility of the food producer

As shown in Figure 11.2, in Chinese characters A is the meaning of 'food', which is composed of the characters of B and C. The characters of B and C mean 'human' and 'clear conscience', which is honesty and credibility. In Chinese therefore, 'food' means something to be eaten made by humans with a clear conscience. There may be many food producers involved in the long chain from farm to table, and they are responsible for food safety. The contamination of Chinese milk by melamine represented a case of food fraud. Melamine was added to mask a milk dilution and pass the protein test. With the improvement of Chinese quality of life, the consumption of dairy products has been increasing at an average of 15% per year since 1995. The Chinese government has also promoted the consumption of milk by children through a school milk scheme since 2000 (China Ministry of Agriculture, 2000). In 2000, the annual production of cow's milk totaled just over 8 million tons. By 2008, this had increased by a factor of 5 to over 36 million tons. Similar growth rates were observed for milk powder products, which increased from less than 1 million tons in 2000 to 4 million tons around 2007 (China Ministry of Agriculture, 2008).

With the increasing trend of dairy consumption, the Chinese government actively supported the growth of the dairy sector with various programs. These included allocation of farmland to raise

食　　人　　良

A   B   C
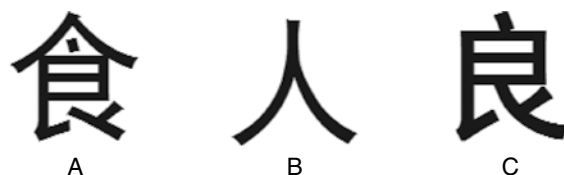
Figure 11.2   Pictorial representation of Chinese characters of food and its components

cattle, improvement of grasslands and yields, and economic incentives such as wavering land-use fees or the provision of discount loans to farmers or processing and packaging operators. As a result, the number of farm holdings has doubled in a very short period of time from 3098 in 2003 to 6478 in 2008. Similarly, the number of enterprises involved in dairy processing, which are mainly located in the regions of Hebei, Henan, Shandong, Heilongjiang, Guangdong, Sichuan, and Shanxi, rose from 355 in 1998 to 717 in 2007 (China Ministry of Agriculture, 2008). These enterprises mainly collected raw milk from personal farmers or milk collection stations. Motivated by greed, some milk collection stations adulterated the product with melamine, an illegal non-food ingredient, to mask milk dilution food fraud.

Honesty and credibility of the food industry is key to safe food. Food safety covers a long chain from farm to table, and the final safe food product is the result of many safe steps. A prerequisite for safe food is food producers with clear consciences. The food and agriculture industry should have a social responsibility to ensure the safety of food products.

11.9.2   Comprehensive and found legislation and regulation system

The melamine incident of 2008 launched a comprehensive reform of the Chinese food safety regime. After the melamine incident, the National People's Congress (NPC) revised the Food Hygiene Law into the Food Safety Law of the People's Republic of China in February 2009, which became effective in June 2009. The new Food Safety Law is the basic law regarding food safety, with the adoption of the risk analysis principle and the 'farm to fork' food chain. Its provisions are set out on the aspects of monitoring and assessment of food safety risks, food safety standards, food production and business operation, food inspection, food import and export control, food safety accidents emergency, supervision and administration, and other related legal liabilities.

Integrating and improving the current national standards of food quality and safety is a very important basis of law enforcement. Under the framework of the Food Safety Law, an action plan of 'Food Safety Standards Integrating and Reviewing' was led by the ministries of Health and Agriculture by integrating the current national food standards in order to set up a comprehensive regulation system. The first session of the Evaluation and Reviewing Committee for National Food Safety Standards was established by the MOH in Beijing on 20 January 2010. Preliminary results include the formulation of 269 new national food safety standards which were published by the end of 2012 (e.g. GB2760 for food additives, GB2761 for mycotoxin, GB2762 for contaminants, and GB2763 for pesticide residues). The 'Usage Standard for Food Additives' (GB2760-2011), in which the use of 2314 kinds of food additives are specified, was successfully revised and published.

Due to the Food Safety Law and Supervision and Management Regulations on Dairy Quality and Safety, the Chinese government reviewed and revised nearly 5000 dairy standards to form a new dairy standard system. This includes more than 160 national dairy standards and aims to correct the problems of repetition, contradiction, and unscientific basis since the end of 2008. On 26 March 2010, the MOH issued 66 new dairy standards including 15 standards on dairy products, 2 standards on good manufacture practice, and 49 standards on detection methods, to form a unified national dairy safety standards system.

However, melamine is neither a raw food ingredient nor a food additive, but an illegal adulteration material that is forbidden from being intentionally added to food. It is therefore on the list of priorities for non-food ingredients as illegal food fraud and food additives frequently abused in food. On 6 April 2011, the MOH issued the interim limit of melamine again. In addition to maximum limit in the National Food Safety Standards, the Chinese government expects to adopt the Good Agricultural Practices (GAP) as well as the Good Manufacturing Practices (GMP) across all food sectors and proceed with the

implementation of Hazard Analysis and Critical Control Point (HACCP) at food production level. The 12th five-year plan for national food safety standards, issued by MOH in 2012, will focus on integrating and cleaning up the current food standards. By 2015, a comprehensive food safety standards system is expected. However, the enactment of legislation is only the first step; effective supervision is also important for food safety.

11.9.3   Effective supervision and risk management

Today, China puts more effort into food safety supervision than in any other country. However, strong supervision does not mean that the administration has hit the right target. In China, supervision of food safety falls within 14 various governmental bodies including the State Food and Drug Administration (SFDA), the Ministry of Agriculture (MOA), the Ministry of Health (MOH), the State General Administration of Quality Supervision, Inspection and Quarantine (AQSIQ), the Ministry of Commerce (MOC), the State Administration of Industry and Commerce (SAIC), and the Ministry of Environmental Protection (MEP). Each of these departments are responsible for a different part of food safety supervision according to relevant laws or regulations, which has reduced the efficiency of the supervision efforts. Problems such as less effective communication, collaboration, and coordination between the agencies may result in less effective supervision, and finally result in loopholes in the chain of 'from farm to fork'.

In practice, this supervision system is a multi-stage management system; it is therefore difficult for collaborations to be set up quickly between departments so that tracing the source of food contamination occurs as soon as possible. For example, in case of the melamine-tainted milk powder in Sanlu, the MOA would be responsible for the original milk quality from the farmer and AQSIQ for the factory in which it is processed. This hihglights a very serious gap between MOA and AQSIQ: who is responsible for the milk collection station? It was the gap in supervision

which led to the melamine incidents, highlighting the importance of integrating the national system of food safety control in China. Fortunately, at the beginning of 2013 NPC approved the State Council to make its reform and transformation of government functions, which focused on food safety first. The China Administration of Food and Drugs (CFDA) has been reorganized to merge the supervision functions of SFDA, AQSIQ, SAIC, and the Office of the State Council Food Safety Commission. Its main responsibility is to implement effective and unified supervision and management on the segment of production, circulation, and consumption of food safety and pharmaceutical safety. The MOA is responsible for the supervision and management of agri-food quality and safety, also merged with that of fixed-point slaughtering of live pigs (originally by MOC). The National Health and Family Planning Commission (NHFPC), comprising the MOH and Population and Family Planning Commission, is responsible for food safety risk assessment and national food safety standards. Finally, the NPC set up the two departments of food safety control enforcement (i.e. SFDA and MOA), and assigned risk assessment, risk management, and policy making (setting of standards based on risk assessment) to NHFPC. The Chinese reform of the national food safety control system will promote China's food safety system to a great extent.

The major framework for national food safety control should be based on risk analysis and a scientifically sound risk assessment system. According to the China Food Safety Law, the function of risk assessment and risk management has been separated from the framework. In October 2011, the China National Center for Food Safety Risk Assessment (CFSA) was inaugurated in Beijing, to provide technical support to the National Expert Committee of Food Safety Risk Assessment established in December 2009. CFSA is responsible for risk surveillance, risk assessment, early warning and risk communication, and scientific support for national food safety standards. It is the first national-level professional body that aims to offer risk assessment to policy makers and the public, and is one of the few interdepartmental

centers in the world, representing an important step by the Chinese government to strengthen food safety (Jia, 2012). Under the technical support of the CFSA, the National Food Safety Risk Monitoring Scheme (NFSRMS) is implemented once a year. The targets supervised include the three aspects of foodborne diseases, chemical contaminants and harmful factors in food, and microorganism pathogens in food. The results obtained by NFSRMS serve the government and the public for food safety risk assessment, food safety standards setting and policy making.

11.9.4   Food safety is the responsibility of the consumer

In the food chain from farm to table, consumers (who are the final link in the chain) are also important for food safety. Firstly, consumers should update their risk perception and understand some basic principles of food safety risk, then act with food safety. In the case of zero tolerance to food fraud with adulteration, consumers should aware that there is no absolute food safety (i.e. 'zero' risk is not possible to achieve). Secondly, consumers should be responsible for food safety at the home level, that is, avoid new food safety risk factors including the introduction of chemical contaminants and microorganisms in the process of food processing in home. Food safety is a combined endeavor of science-based risk assessment, the honesty of the food producer, comprehensive legislation and regulation of the nation, effective supervision and management of the government, and understanding from the consumer's point of view.

11.10   Conclusions and future perspectives

These two incidents of melamine contamination (pet food and infant formula) have initiated a new era of national food safety control in China to ensure food safety is the responsibility of food producers, the government, and the consumers. Honesty and credibility of the food industry is key to safe food. Food safety covers a long chain from farm to table, and the final safe food product is the result of many safe steps. In the same way as other nations, China still has a long way to go before being able to guarantee safe food.

Advancements made in analytical chemistry, toxicology, exposure, and risk analysis of melamine in food have been fully explored worldwide. Based on these outcomes, the food safety system in China has been reformed. Food safety is defined by the ethics of food producers throughout the whole supply chain. If criminal engineers insist on adulterating food products to evade existing quality assurance (QA) system, the QA system will react by developing new tests. However, the next adulterant is still unknown.

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12
Heavy Metals of Special Concern to Human Health and Environment

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Summary

Heavy metals are natural components of the Earth's crust and their excessive release in the environment is mainly due to human anthropogenic activities. These heavy metals cannot be degraded or destroyed and can easily enter the human body along the foods chains (via food, water, or air). Moreover, they have a tendency to accumulate in the human body, causing detrimental health effects even at minute concentrations. In this respect, heavy metals of special concern to human health and environment will be addressed.

12.1   Introduction

The main threats to human health and environment from heavy metals are mainly associated with exposure to certain metals such as mercury, cadmium, lead, chromium, and arsenic. These metals have been extensively studied and their effects on human health regularly reviewed by international bodies such as the World Health Organization (WHO). Humans are responsible for the release of large quantities of metals, including arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) into the environment (Nriagu and Pacyna, 1988). Once released, these metals are cycled between environmental media. Soils may become highly contaminated due to both direct land disposal of wastes and fallout from atmospheric emissions.

Heavy metals in particular have long residence times in soils because of sorption to soil particles (Fetter, 1992), which increases the potential risks for human exposure and adverse human health effects at both the cellular level (Delmas *et al.*, 2000; Fulladosa *et al.*, 2002) as well as higher levels (Stewart *et al.*, 2003). Metal exposure to humans has been widely studied. Different populations show disproportionate susceptibilities to metals (Vahter *et al.*, 2002). Infants and children are particularly susceptible to neurotoxicological damage from metal exposure due to their ongoing rapid intellectual development. The neurological systems of fetuses, often the target of damage from metals, are also at risk because of the transfer of maternal blood in which metals may bioaccumulate (Calderón *et al.*, 2003). Arsenic, chromium, and lead are three metals of particular concern for neurological impairment. A strong association was found between lower intelligence scores in Bangladeshi children and arsenic concentrations in drinking water after adjusting for social and economic factors (Wasserman *et al.*, 2004). Chromium exposure, particularly from ingestion, can result in neurotoxic effects (Stewart *et al.*, 2003). Thornton *et al.* (1990) found that lead blood levels of children were correlated with soil and dust lead levels in and around the home. Environmental lead exposure of pregnant women in Yugoslavia was also associated with lower intelligence test scores of their children (Factor-Litvak *et al.*, 1999). Most exposure to metals is associated with contaminated groundwater and soils (Caussy *et al.*, 2003).

Rana (2008) reviewed the induction of apoptosis by heavy metals. Apoptosis, also known as programmed cell death, is a highly regulated and crucial process found in all multicellular organisms. It is not only implicated in regulatory mechanisms of cells, but has been attributed to a number of diseases, i.e. inflammation, malignancy, autoimmunity, and neurodegeneration. A variety of toxins can induce apoptosis. Carcinogenic transition metals, namely cadmium, chromium, and nickel, promote apoptosis along with DNA base modifications, strand breaks, and rearrangements. The generation of reactive oxygen species, accumulation of Ca^{2+}, upregulation of caspase-3, down-regulation of bcl-2, and deficiency of p-53 lead to arsenic-induced apoptosis. In the case of cadmium, metallothionein expression determines the choice between apoptosis and necrosis. Reactive oxygen species (ROS) and p-53 contribute in apoptosis caused by chromium. Immunosuppressive mechanisms contribute in lead-induced apoptosis whereas in the case of mercury, p-38 mediated caspase activation regulate apoptosis. Nickel kills the cells by apoptotic pathways. Copper induces apoptosis by p-53-dependent and -independent pathways. Beryllium stimulates the formation of ROS that play a role in Be-induced macrophage apoptosis. Selenium induces apoptosis by producing superoxide that activates p-53. Disorders of apoptosis may therefore play a critical role in some of the most debilitating metal-induced afflictions including hepatotoxicity, renal toxicity, neurotoxicity, autoimmunity, and carcinogenesis. An understanding of metal-induced apoptosis will be helpful in the development of preventive molecular strategies (Rana, 2008).

In the following sections, more emphasis is placed on the effects of heavy metals (e.g. Hg, Cd, Pb, Cr, As) on health and environment (Sections 12.2–12.7, respectively); other metals are addressed in lesser detail in Section 12.8.

12.2   Mercury

12.2.1   Occurrence, use and exposure

Mercury (Hg) is a global pollutant with complex and unusual chemical and physical properties. The major natural source of mercury is the degassing of the Earth's crust, emissions from volcanoes, and evaporation from natural bodies of water. Worldwide mining of the metal leads to indirect discharges into the atmosphere. The usage of mercury is widespread in industrial processes and in various products (e.g. batteries, lamps, and thermometers). It is also widely used in dentistry as an amalgam for fillings and by the pharmaceutical industry. Concern over mercury in the environment arises from the extremely toxic forms in which mercury can occur (Anonymous, 2003).

Mercury demonstrates the diversity of effects by its different chemical species. It is the only metal that exists in a liquid state at room temperature. The vapor is much more hazardous than liquid form. The element exists in three oxidation states. In the zero oxidation state (Hg), mercury exists in metabolic form or as the vapor. The mercurous and mercuric are the higher oxidation states where the mercury has lost one atom (Hg+) and two electrons (Hg^2+), respectively. In addition, mercuric mercury can form a number of stable organic mercury compounds by attaching to one or two carbon compounds. Methyl mercury (CH$_3$Hg$^+$) is the most important organic form from the human health point of view. Different forms of mercury enter into the environment through different pathways. The vapor from metallic mercury is readily absorbed in the lungs. The dissolved form is readily absorbed by the bloodstream and diffuses to all tissues in the body (Anonymous, 2003).

Mercury is mostly present in the atmosphere in a relatively unreactive form as a gaseous element. The long atmospheric lifetime (of the order 1 year) of its gaseous form means the emission, transport, and deposition of mercury is a global issue. Natural biological processes can cause methylated forms of mercury to form which bioaccumulate over a million-fold and concentrate in living organisms, especially fish. These forms of mercury, monomethylmercury and dimethylmercury, are highly toxic, causing neurotoxicological disorders. The main pathway for mercury to humans is through the food chain and not by inhalation (Anonymous, 2003).

Mercury enters our food supply through several sources; the first is from contaminated water supply inhabited by fish that are later consumed by humans. Mercury concentrations in fish significantly exceed the concentrations in the water. Meat can also contain mercury, after environmental pollution of fields that livestock use for grazing. Mercury is not commonly found in plant products, but it can enter human bodies through vegetables and other crops when sprays that contain mercury are applied in agriculture (Anonymous, 2003).

As a result of increased transport of mercury to lakes, concentrations in fish increased during the last century. In southern parts of Finland, Norway, and Sweden, the mercury content in a 1 kg pike (*Esox lucius*) is about 0.5–1.0 mg kg^{-1}; the natural background value is estimated at about 0.2 mg kg^{-1} (Rognerud *et al.*, 1996; Johansson *et al.*, 2001). The recommended limit of 0.5 mg kg^{-1} is exceeded for 1 kg pike in about 50% of the lakes in Sweden and in 85% of the lakes in southern and central Finland (Lindqvist *et al.*, 1991).

12.2.2 Health effects

Mercury is a toxic substance which has no known function in human biochemistry or physiology and does not occur naturally in living organisms. Inorganic mercury poisoning is associated with tremors, gingivitis and/or minor psychological changes, together with spontaneous abortion and congenital malformation. Monomethyl mercury causes damage to the brain and the central nervous system, while foetal and postnatal exposure have given rise to abortion, congenital malformation, and development changes in young children (Anonymous, 2003).

Gastrointestinal absorption of mercury occurs through contaminated food. Inorganic mercury shows the highest affinity with the kidney whereas organic mercury has greater affinity for the brain. Excretion of mercury from the body is by way of urine and feces. All forms of mercury cross the placenta to the fetus as known in experimental animals (Goyer, 1996). Unborn children (i.e. fetuses) are therefore the most susceptible population group, the exposure being mainly from fish in the diet of the mother. Methyl mercury is also excreted in mothers' milk. Human biomonitoring and diet-modeling data indicate that tolerable dietary intakes of methyl mercury are exceeded among subpopulations that consume large amounts of fish, e.g. in Scandinavia, North America, and France. For several species of (mainly large predatory) freshwater and marine fish and mammals, a mercury level of 0.5 mg kg^{-1} (the value used as a guideline in many countries) is often exceeded (WHO/Europe, 2007).

12.2.3 Toxicology of mercury

Acute exposure to inorganic mercury may give rise to lung damage. Chronic poisoning is characterized by neurological and psychological symptoms such as tremor, changes in personality, restlessness, anxiety, sleep disturbance, and depression. The symptoms are reversible after cessation of exposure. Because of the blood–brain barrier there is no central nervous involvement related to inorganic mercury exposure. Metallic mercury may cause kidney damage, which is reversible after exposure has stopped. It has also been possible to detect proteinuria at relatively low levels of occupational exposure. Metallic mercury is an allergen, which may cause contact eczema, and mercury from amalgam fillings may give rise to oral lichen. It has been feared that mercury in amalgam may cause a variety of symptoms. This so-called 'amalgam disease' is however controversial, and although some authors claim proof of symptom relief after removal of dental amalgam fillings (Lindh *et al.*, 2002); there is no scientific evidence of this (Langworth *et al.*, 2002). Mercury exposure affects the central nervous system, brain functions, causes DNA and chromosomal damage, and causes reproductive problems. The most common route of human exposure to mercury is through environmental contamination of our food source (Anonymous, 2003).

Organic mercury (Methyl mercury) poisoning has latency of 1 month or longer after acute exposure, and the main symptoms relate to nervous system damage (Weiss *et al.*, 2002). The earliest symptoms are parestesias and numbness in the hands and feet. Later, coordination difficulties and concentric constriction of the visual field may develop as well as auditory symptoms. High doses may lead to death, usually 2–4 weeks after onset of symptoms. The Minamata catastrophe in Japan in the 1950s was caused by methyl mercury poisoning from fish contaminated by mercury discharges to the surrounding sea. In the early 1970s, more than 10,000 persons in Iraq were poisoned by eating bread baked from mercury-polluted grain, and several thousand people died as a consequence of the poisoning. However, the general population does not face significant health risks from methyl mercury exposure with the exception of certain groups with high fish consumption (Järup, 2003).

A high dietary intake of mercury from consumption of fish has been hypothesized to increase the risk of coronary heart disease (Salonen *et al.*, 1995). When other risk factors for coronary heart disease had been controlled for, mercury levels were not significantly associated with the risk of coronary heart disease (Yoshizawa *et al.*, 2002).

In a recent control study, the joint association of mercury levels in toenail clippings and docosahexaenoic acid levels in adipose tissue with the risk of a first myocardial infarction in men was evaluated (Guallar *et al.*, 2002). Mercury levels in the patients were 15% higher than those in controls (95% CI, 5–25%), and the adjusted odds ratio for myocardial infarction associated with the highest compared with the lowest quintile of mercury was 2.16 (95% CI, 1.09–4.29; *P* for trend = 0.006).

12.3 Cadmium

12.3.1 Occurrence, use and exposure

Cadmium (Cd) has many applications to its credit. It is mainly used as a color pigment in paints and plastics. The most significant use of cadmium is in nickel/cadmium batteries as rechargeable or secondary power sources exhibiting high output, long life, low maintenance, and high tolerance to physical and electrical stress. Cadmium coatings provide good corrosion resistance, particularly in high-stress environments such as marine and aerospace applications where high safety or reliability is required; the coating is preferentially corroded if damaged. Other uses of cadmium are as pigments, stabilizers for PVC, in alloys, and in electronic compounds. Cadmium is also present as an impurity in several products, including phosphate fertilizers, detergents, and refined petroleum products. It is a byproduct of zinc and lead mining and smelting, which are important sources of environmental pollution (Anonymous, 2003).

Cadmium exists in low concentrations in all soils. It is actively extracted from its ores for commercial purposes and is also emitted in industrial processes such as metal melting and refining, coal- and oil-fired power stations, electroplating plants, etc. Cadmium is released into the atmosphere by natural and anthropogenic means. Volcanoes, windborne particles, and biogenic emissions are considered the main natural sources of cadmium in the atmosphere (Nriagu, 1989). The anthropogenic sources of cadmium include non-ferrous metal production, stationary fossil fuel combustion, waste incineration, iron and steel production, and cement production. It is spread by air and water (sewage sludge) far over sea and land, but especially in the vicinity of heavy industrial plants. Cadmium is today regarded as the most serious contaminant of the modern age. It is absorbed by many plants and sea creatures and, because of its toxicity, presents a major problem for foodstuffs. Contamination through fertilizers becomes an increasing problem. Unlike lead, cadmium contamination cannot be removed from plants by washing them; it is distributed throughout the organism. It is often difficult to be certain of the cause of a cadmium content found in fruit or vegetables, as the substance in its natural form exists everywhere in the soil and is absorbed by the roots (Anonymous, 2003).

Annual inputs from long-range transboundary air pollution (LRTAP) and mineral and organic fertilizers to topsoil are roughly of the same magnitude. They all continue to contribute to the existing (relatively large) accumulation of cadmium in the topsoil (WHO/Europe, 2007). Despite the decrease in cadmium emissions, ambient air concentrations, and deposition, recently published data do not show a decrease in the cadmium body burdens in non-smokers in the last decade. Studies on the cadmium balance in the top layers of arable soils indicate that the input of this heavy metal still exceeds its removal. Cadmium is accumulating in soils and catchments under certain environmental conditions, thus increasing the risk of future exposure through food. In view of the narrow margin of safety, every effort should therefore be made to make further reductions regarding cadmium emission into the atmosphere and other types of cadmium input into soil (WHO/Europe, 2007).

12.3.2   Health effects

Cadmium derives its toxicological properties from its chemical similarity to zinc which is an essential micronutrient for plants, animals, and humans. Once cadmium is absorbed by an organism, it remains resident for many years (over decades) although it is eventually excreted. In humans, long-term exposure is associated with renal dysfunction. Cadmium may also produce bone defects (osteomalacia, osteoporosis) in humans and animals. In addition, the metal can be linked to increased blood pressure and effects on the myocardium in animals, although most human data do not support these findings (WHO/Europe, 2007).

Unlike mercury and lead, human exposure to cadmium is most always through food. Food is the main source of cadmium exposure in the general population (representing >90% of the total intake in non-smokers). In heavily contaminated areas, dust re-suspension can constitute a substantial part of the crop contamination and exposures via inhalation and digestion. Exposure to humans also arises through cadmium in ambient air and drinking water. Additionally, cadmium is present in cigarette smoke, which acts as a blood transport mechanism for cadmium into the lungs. It can also travel via the blood into the liver and then into the kidneys (Anynomous, 2003).

Cigarette smoking represents an additional source of cadmium, which may exceed that from food. One cigarette contains about $1–2\,\mu g$ cadmium. An average of about 10% of this is inhaled during smoking. It can therefore be estimated that a person smoking 20 cigarettes per day will absorb about $1\,\mu g$ of cadmium (Järup *et al.*, 1998). According to Erzen and Kragelj (2006), the median blood cadmium (B-Cd) concentration in Slovenia was $0.5\,\mu g\,L^{-1}$ in non-smokers, $1.0\,\mu g\,L^{-1}$ in light to moderate smokers (less than 20 cigarettes/day) and $1.5\,\mu g\,L^{-1}$ in heavy smokers (more than 20 cigarettes/day).

Kidney and bone are the critical target organs with regard to environmental exposure. The main critical effects include increased excretion of low-molecular-weight proteins in the urine (as a result of proximal tubular cell damage) and an increased risk of osteoporosis. An increased risk of lung cancer has also been reported following inhalation exposure in occupational settings. The margin of safety between the present daily intake of cadmium in the diet and the intake that can result in effects is very narrow and, for highly exposed subpopulations, even non-existent. Population groups at risk include the elderly, people with diabetes, and smokers. Women may be at increased risk because they have lower iron stores than men and, consequently, absorb more cadmium at the same level of exposure (WHO/Europe, 2007).

12.3.3   Cadmium toxicology

The toxicology of cadmium has been reviewed by Friberg *et al.* (1985), WHO (1992a), EPA (1997), and ATSDR (1998). Acute toxicity may result from the ingestion of relatively high concentrations of cadmium through contaminated beverages and food. The long-term effects of low-level exposure to cadmium are chronic obstructive pulmonary disease and emphysema and renal tubular disease. There may also be effects on the cardiovascular and skeletal systems. Epidemiologic studies have shown a relationship between occupational exposure to cadmium and lung cancer and possibly prostate cancer. Cadmium has been identified by the International Agency for Research on Cancer as a Category 1 human carcinogen (IARC, 1993b).

12.3.3.1   Exposure via the gastrointestinal tract
For non-smokers, food constitutes the principal environmental source of cadmium via the gastrointestinal tract. In recent years, the mean daily intake of cadmium from food amounted to 17.3 µg in Croatia (Sapunar-Postruznik *et al.*, 1996), 11–19 µg in the Czech Republic (Puklova *et al.*, 2005), 27 µg in France (Biego *et al.*, 1998), 10–14 µg in Germany (Muller *et al.*, 1998), 23.3 µg in Poland (in females) (Marzec and Schlegel-Zawadska

2004), 11–29 µg in Spain (Rubio *et al.*, 2006), and 11–16 µg in Sweden (Berglund *et al.*, 1994).

Dietary cadmium intake is log-normally distributed; a small increase in the population's average daily intake of cadmium will therefore result in a much larger increase in the fraction of the population having the highest intake. An increase in the median daily intake by a factor of 2 (i.e. from 15 to 30 µg day^{-1}) would correspond to an increase in the 95th percentile from about 20 to 60 µg day^{-1} (Järup *et al.*, 1998). The provisional tolerable weekly intake (PTWI) set by WHO and the Food and Agriculture Organization of the United Nations (FAO) (WHO/FAO, 1993) for cadmium is 500 µg (a weekly intake of 7 µg kg^{-1} body weight), corresponding to a daily intake of 70 µg or 1 µg kg^{-1} bw day^{-1}. The USEPA Reference Dose amounts to 1 µg kg^{-1} bw day^{-1} in food and 0.5 g kg^{-1} bw day^{-1} in drinking water (USEPA, 1999). These values are based on the chronic effects of cadmium on kidney function. However, it has been suggested that the PTWI should be lowered (Järup *et al.*, 1998; Nordberg, 1999).

12.3.3.2   Relevance of various routes of exposure
There are three main anthropogenic sources of terrestrial cadmium: atmospheric deposition, agricultural application of phosphate fertilizers, and use of municipal sewage sludge as a fertilizer on agricultural soils. It has been reported that 90% of the cadmium in soil remains in the top 15 cm (ATSDR, 1999a). Accumulation of cadmium in the soil depends on the soil properties, with clay soils generally retaining more cadmium than sandy soils.

A comparison of cadmium intake and uptake via respiratory and dietary routes in China and Japan was presented by Zhang *et al.* (1997). On average, the cadmium concentration in air was 7.3 ng m^{-3} in China, the daily exposure via the respiratory tract was calculated to be 0.11 µg and the uptake (50% absorption) was 0.05 µg. The daily intake via food was reported to be 9.9 µg and the uptake (7.5% absorption) was 0.74 µg day^{-1}. In Japan, uptakes via the respiratory and food routes were calculated to be 0.07 and 2.41 µg day^{-1}, respectively. The uptake via food was estimated

to amount to 93.7% in China and 97.2% in Japan. In the Czech Republic, the daily intakes via the respiratory route, water and food were estimated to be 0.01 µg day^{-1}, 0.17 µg day^{-1} and 18.2 µg day^{-1} (0.05%, 0.92% and 99.3%), respectively (Kliment, 1996).

In Canada, the estimated daily cadmium intakes in adults over 20 years (assumed to weigh 70 kg, to breath 23 m^3 of air, drink 0.4 L of water, ingest 20 mg of soil, and to smoke 20 cigarettes per day) were as follows: from air the intake was 0.33–1.3 ng kg^{-1} bw day^{-1}; from drinking water it was <0.057–0.51 ng kg^{-1} bw day^{-1}; from food it was 210 ng kg^{-1} bw day^{-1}; from soil it was 0.16–0.33 ng kg^{-1} bw day^{-1}; and from cigarettes it was 53 ng kg^{-1} bw day^{-1} (Newhook et al., 1994).

At a cadmium concentration of 1 mg kg^{-1} soil, the intake of cadmium from ingestion of soil would be approximately 0.05–0.2 µg day^{-1}, assuming a soil-ingestion rate of 0.05–0.2 g day^{-1} for adults (Choudhury et al., 2001).

12.3.3.3 Toxic effects
Cadmium is concentrated particularly in the kidneys, the liver, the blood-forming organs, and the lungs. It most frequently results in kidney damage (necrotic protein precipitation) and metabolic anomalies caused by enzyme inhibitions. It is now known that the Itai-Itai sickness in Japan (with bone damage) is a result of the regular consumption of highly contaminated rice. Cadmium, like lead, is a cumulative poison, i.e. the danger lies primarily in the regular consumption of foodstuffs with low contamination. In contrast to lead however, the definition of an exact toxicity limit is not possible for cadmium. The decisive point is whether absorption of the existing cadmium actually takes place. This is dependent upon (1) the composition of the diet as a whole and (2) the bio-availability of the cadmium compound present (WHO/Europe, 2007).

12.3.3.4 Toxicokinetics
Pulmonary absorption of inhaled cadmium falls within the range 10–50% (WHO/IPCS, 1992). The average normal gastrointestinal absorption of ingested cadmium in humans falls within the range 3–7%. Cadmium in the tissues is mainly bound to metallothionein. The synthesis of this protein probably represents the body's defense mechanism against the toxic cadmium ion. Liver and kidney tissues are the two main sites of cadmium storage. The newborn infant is virtually free of cadmium but, over a lifetime, these organs accumulate considerable amounts of cadmium (about 40–80% of the body burden). Important health endpoints include kidney and bone damage and cancer. The kidney is the critical organ with regard to long-term occupational and environmental exposure to cadmium, and all health-based recommendations relate to the early disturbance of renal function (Järup et al., 1998).

In low-level environmental exposures, about 30–50% of the cadmium body burden is stored in the kidneys. Cadmium elimination from blood has been described in an open two-compartment model as having a fast-decay half-time of 15–120 days and a slow-decay half-time of 7.4–16 years (Järup et al., 1983). Cadmium is eliminated in urine and faeces; daily faecal and urinary excretion is estimated to constitute 0.007% and 0.009% of the body burden, respectively (ATSDR, 1999a).

12.3.3.5 Bones and calcium metabolism
The available data show that cadmium can affect calcium and phosphorus metabolism generally, both in industrial workers and in people exposed in the general environment. Painful bone disorders, including osteomalacia, osteoporosis, and spontaneous bone fracture, have been observed in humans chronically exposed to cadmium in food. In the Jinzu river basin in Japan, exposure to cadmium was caused by contamination of river water. Osteomalacia most often affects women with several risk factors such as poor nutrition and multiparity (WHO/IPCS, 1992).

12.3.3.6 Cancer
In its latest evaluation of the carcinogenic risk from cadmium exposure, IARC (1993a) concluded that there was sufficient evidence to classify cadmium and cadmium compounds as human carcinogens (Group I). This assessment was, to a great extent, dependent on the significant relationship between the risk of lung cancer and estimated

cumulative exposure to cadmium reported by Thun *et al.* (1985) and Stayner *et al.* (1992) in their analyses of mortality in a cohort of workers from a single cadmium recovery plant in the United States. On the basis of this analysis, the lifetime excess of lung cancer at $100\,\mu g\,m^{-3}$ of cadmium fumes would be approximately 50–111 lung cancer deaths per 1000 workers. Sorohan and Lancashire (1997) performed a re-analysis of this cohort, with the inclusion of data on arsenic exposure. The results of this re-evaluation indicate that cadmium is carcinogenic only under conditions of a concomitant exposure to arsenic. The evidence for cadmium as a human carcinogen is rather weak. EU has classified cadmium to carcinogen category 2, USEPA (1999) to category B1, and ACGIH (2005) to category A2.

12.4   Lead

12.4.1   Occurrence, use and exposure

Lead (Pb) is a ubiquitous metal; it can be detected in all inert environments and in all biological systems. Lead has been mined since ancient times and has been processed in many ways, e.g. for water pipes, containers, and even for sweetening wine as acetate ('lead sugar'). It has a variety of applications from use in pipes and paints to its use in pesticides and a number of other applications. Lead is among the most recycled non-ferrous metals and its secondary production has therefore grown steadily despite declining lead prices. Its physical and chemical properties are applied in the manufacturing, construction, and chemical industries. It is easily shaped and is malleable and ductile. There are eight broad categories of use: batteries, petrol additives (no longer allowed in the EU), rolled and extruded products, alloys, pigments and compounds, cable sheathing, shot, and ammunition. World production amounts to millions of tons and is used in the manufacture of accumulators, solders, pigments, cables, and anti-rust agents (red lead/lead oxide), and still a considerable amount into anti-knock petrol (Anonymous, 2003).

The principal routes of exposure in general population are air, water, food, lead-glazed pottery, industrial emissions and dust. Other potential sources of exposure to lead are recreational shooting, hand loading of ammunition, soldering, jewelry making, pottery making, gunsmithing, glass polishing, painting, and stained glass crafting. Children absorb a greater proportion of lead than adults. More than 90% of the lead in blood is in red blood cells. The largest and kinetically slowest pool is the skeleton. The largest soft tissue accumulations of lead occur in the liver and kidney but it may be found in most of the tissues of the body. The major route of its excretion is the kidney (EPA, 1986; WHO, 1992b; Goyer, 1993; NRC, 1993; ATSDR, 1999b).

Lead is released into the atmosphere from natural and anthropogenic sources. Natural emissions are from wind resuspension and from sea salt, volcanoes, forest fires, and biogenic sources (Nriagu, 1989). According to Nriagu (1989), these emissions are not entirely natural but contain some contributions from historical depositions of anthropogenic lead. Major anthropogenic emission sources of lead on a global scale include the combustion of fossil fuels from, for example, traffic, non-ferrous metal production, and iron and steel production. Some contributions are also made by cement production and waste disposal (Pacyna and Pacyna, 2001).

12.4.2   Health effects

In humans exposure to lead can result in a wide range of biological effects depending on the level and duration of exposure. Various effects occur over a broad range of doses, with the developing foetus and infant being more sensitive than the adult. High levels of exposure may result in toxic biochemical effects in humans which in turn cause problems in the synthesis of haemoglobin, effects on the kidneys, gastrointestinal tract, joints and reproductive system, and acute or chronic damage to the nervous system (Anonymous, 2003).

Average daily lead intake for adults in the UK is estimated at $1.6\,\mu g$ from air, $20\,\mu g$ from drinking water and $28\,\mu g$ from food. Although most people

receive the bulk of their lead intake from food, in specific populations other sources may be more important such as water in areas with lead piping and plumb-solvent water, air near point of source emissions, soil, dust, paint flakes in old houses, or contaminated land. Lead in the air contributes to lead levels in food through deposition of dust and rain containing the metal on crops and the soil. For the majority of people in the UK, however, dietary lead exposure is well below the provisional tolerable weekly intake recommended by the UN Food and Agriculture Organization and the World Health Organization (WHO, 1992b).

12.4.3   Lead toxicology

12.4.3.1   Relevance of various routes of exposure

The relative contributions of sources of pollution differ depending on local conditions. Food is the predominant source of lead uptake in the general population. Ingestion of contaminated soil, dust, and old lead-based paint due to hand-to-mouth activities may also be important regarding lead intake in infants and young children. When tap water systems with leaded pipes are used, lead intake via drinking water can be an important source, especially in children. Inhalation exposure may be significant when lead levels in the air are high (WHO/Europe, 2007).

Lead levels in ambient air have decreased in recent decades: between 1990 and 2003, lead levels in air in Europe fell by 50–70%. Similar decreases have been observed for atmospheric deposition. The annual lead inputs from long-range transboundary air pollution (LRTAP) and from the addition of organic and inorganic fertilizers to topsoil are roughly similar in magnitude, depending on the country and the agricultural activity. Those inputs are relatively small in comparison to the existing accumulations, natural sources and resuspension. However, LRTAP may contribute significantly to the lead content of crops through direct deposition. Although uptake via plant roots is relatively small, rising lead levels in soils over the long term are a matter of concern and should be avoided because of the possible health risks of low-level exposure.

Lead emissions to the atmosphere should therefore be kept as low as possible (WHO/Europe, 2007; Yoon *et al.*, 2008).

12.4.3.2   Toxic effects

Lead can trigger both acute and chronic symptoms of poisoning. Acute intoxications only occur through the consumption of relatively large single doses of soluble lead salts. Chronic intoxications can arise through the regular consumption of foodstuffs only slightly contaminated with lead. Lead is a typical cumulative poison. The danger of chronic intoxications is the greater problem.

Basically, as a result of their comparatively high affinity for proteins, the lead ions consumed bond with the hemoglobin (red blood pigment) and the plasma protein of the blood. This leads to inhibition of the synthesis of red blood cells and thus of the vital transport of oxygen. If the bonding capacity here is exceeded, lead passes into the bone-marrow, liver, and kidneys. Such intoxication leads to: encephalopathies in the central nervous system (CNS); disturbances in kidney and liver functions progressing as far as necrosis; damage to the reproductive organs; and anaemias and many metabolic deficiency symptoms.

Some of the injurious processes are still not properly understood. Particularly dangerous to all forms of life are the organic lead compounds. They cause injuries to mental development such as reduction of intelligence, growth disturbances, and plasticity. In animal experiments, the consumption of domestic and surface dust leads to a measurably increased heavy metal content in the blood. Little is known about the excretion of lead once it has been absorbed. The greatest part accumulates in the body. Lead is not considered to be a carcinogen or mutagen (Anonymous, 2003).

12.4.3.3   Toxicokinetics

Problems for foodstuffs were caused for a long time, and are still caused today on occasion, by the soldered seams of cans and the soldered closures of condensed milk cans, the metal caps of wine bottles and, still, by lead pipes in drinking water systems.

Lead primarily acts by competing with endogenous cations on protein binding sites. In particular, lead can substitute both calcium and zinc in numerous proteins (Goering, 1993; Goldstein, 1993; Simons, 1993; Zawia *et al.*, 1998). This substitution can further alter the normal functioning of these proteins and can therefore alter the cellular pathways and induce aberrant gene transcription (Zhu and Thiele, 1996; Bouton and Pevsner, 2000). Earlier, Bouton *et al.* (2001) demonstrated that the expression of numerous genes can be altered by lead.

Lead can enter the human body through uptake of food (65%), water (20%), and air (15%). Many foodstuffs, including fruit, vegetables, meat, grains, seafood, soft drinks, and wine, contain lead. Cigarette smoke also contains small amounts of lead. Lead can enter (drinking) water through corrosion of pipes; this is more likely to happen when the water is slightly acidic. (This is why public water treatment systems are now required to carry out pH-adjustments of drinking water.) Lead causes many problems in the human body upon bioaccumulation such as disruption of blood synthesis, kidney damage, high blood pressure, miscarriage and low sperm count (Anonymous, 2003).

12.4.3.4 Vulnerability of children to lead poisoning

Children are particularly at risk from lead consumption, both before and after birth, as they absorb lead more rapidly than adults. Particularly affected are small children with their habit of placing dirty fingers and objects of all kinds into their mouths or licking them (so-called mouth/hand activity) and, in this way, swallowing dust and soil particles containing heavy metals, for example from lead-based paints.

Lead is a well-known neurotoxin. Impairment of neurodevelopment in children is the most critical effect. Exposure in utero, during breastfeeding, and in early childhood may all be responsible for the effects. Lead accumulates in the skeleton, and its mobilization from bones during pregnancy and lactation causes exposure to fetuses and breastfed infants. The lifetime exposure of woman before pregnancy is therefore important (WHO/Europe, 2007; Yoon *et al.*, 2008).

Epidemiological studies consistently show that effects in children are associated with blood lead (B-Pb) levels of about 100–150 μg L^{-1}. There are indications that lead is harmful even at B-Pb concentrations considerably below 100 μg L^{-1}; there may be no threshold for these effects. In many areas there have been major decreases in B-Pb levels in recent decades, mainly because of the phasing out of leaded petrol but also because of reductions in other sources of exposure. At present, the lowest average B-Pb level in several European countries is about 20 μg L^{-1}, but reliable B-Pb information from many parts of Europe is lacking (WHO/Europe, 2007).

Lead poisoning, which is so severe as to cause evident illness, is now very rare indeed. At intermediate concentrations, however, there is persuasive evidence that lead can have small, subtle, subclinical effects, particularly on neuropsychological developments in children. Some studies suggest that there may be a loss of up to 2 IQ points for a rise in blood lead levels from 10 to 20 μg dL^{-1} in young children (Anonymous, 2003). Lead toxicity involves several organ systems. The critical effects in infants and children involve the nervous system (ASTDR, 1999b). However, in the adult population it is hypertension. Effects on the heme system are treated as biochemical indicators of exposure to lead. Other target organs are gastrointestinal, reproductive, and skeletal systems. Lead nephropathy is one of the oldest recognized health effects of lead (Oliver, 1914). Lead is a renal carcinogen in rodents. Microscopical examination of kidney shows dense, homogeneous eosinophilic intranuclear inclusions. There appears to be no specific biomarker for lead-induced renal disease. Lead has been found to be immunosuppressive (McCabe and Lawrence, 1991). Gametotoxic effects have been demonstrated in male and female animals (Stowe and Goyer, 1971). Epidemiologic studies suggest a relationship between occupational lead exposure and cancer of the lung and brain.

12.5 Chromium

12.5.1 Occurrence, use and exposure

Chromium (Cr) is used in metal alloys and pigments for paints, cement, paper, rubber, and other materials. Low-level exposure can irritate the skin and cause ulceration. Long-term exposure can cause kidney and liver damage, and damage to circulatory and nerve tissue. Chromium often accumulates in aquatic life, adding to the danger of eating fish that may have been exposed to high levels of chromium. Chromium occurs in different oxidation states ranging from Cr2+ to Cr6+ but only trivalent and hexavalent forms are of biological significance. The trivalent is the more common form; however, hexavalent forms such as chromate compounds are of greater industrial importance (Yoon *et al.*, 2008).

12.5.2 Health effects

Health effects of chromium have been reviewed from time to time (Fishbein, 1981; WHO, 1988; O'Flaherty, 1995). Systemic toxicity occurs from ingestion of high amounts of Cr (VI). Low-level exposure causes glomerular and tubular damage. Cr (VI) is corrosive and causes chronic ulceration of skin surface. Asthma may be caused by occupational exposure to Cr (Bright *et al.*, 1997). Known toxic effects of chromium in human have been attributed to Cr (VI) where it is reduced to Cr (III) that complexes with intracellular macromolecules. Exposure to chromium, particularly in the chrome production and chrome pigment industries, is associated with diseases of the respiratory tract (Langard and Norseth, 1986).

The mechanism of Cr (VI) carcinogenicity in the lung is believed to be its reduction to Cr (III) and to generation of reactive intermediates. Cr elicits a variety of effects: (1) at the biochemical level: the formation of coordination covalent interaction of Cr (II) and Cr (III) with DNA and of DNA–DNA and DNA–protein complexes; (2) at the genomic level: the induction of gene expression (oxidant stress, metallothionein and tumor suppressor genes), gene mutations, DNA lesions, inhibition of protein synthesis and arrest of DNA replication; and (3) at the cellular level: cell cycle arrest, apoptosis, and neoplastic transformations (Bridgewater *et al.*, 1998; Dubrovskaya and Wetterhahn, 1998; Singh *et al.*, 1998; Kaltreider *et al.*, 1999; Solis-Heredia *et al.*, 2000). DNA–protein complexes may serve as biomarkers of exposure. There exists conclusive evidence that chromium compounds cause cancer at sites other than the respiratory tract.

12.6 Arsenic

12.6.1 Occurrence, exposure and dose

Arsenic (As) is a naturally occurring metalloid, widely distributed and occurring in rock, soil, water, and air. Inorganic arsenic is present in groundwater used for drinking in several countries all over the world (e.g. Bangladesh, Chile, and China), whereas organic arsenic compounds (such as arsenobetaine) are primarily found in fish, giving rise to possible human exposure (WHO, 2001). Smelting of non-ferrous metals and the production of energy from fossil fuel are the two major industrial processes that lead to arsenic contamination of air, water and soil, smelting activities being the largest single anthropogenic source of atmospheric pollution (Chilvers and Peterson, 1987). Other sources of contamination are the manufacture and use of arsenical pesticides and wood preservatives.

The working group of the EU DG Environment concluded that there were large reductions in the emissions of arsenic to air in several member countries of the European Union in the 1980s. In 1990, the total emissions of arsenic to the air in the member states were estimated to be 575 tons. In 1996, the estimated total releases of arsenic to the air in the UK were 50 tons (DG Environment, 2000). Concentrations in air in rural areas range from <1–$4\,ng\,m^{-3}$, whereas concentrations in cities may be as high as $200\,ng\,m^{-3}$. Much higher concentrations ($>1000\,ng\,m^{-3}$) have been measured

near industrial sources. Water concentrations are usually $<10\,\mu g\,L^{-1}$, although higher concentrations may occur near anthropogenic sources. Levels in soils usually range from 1 to $40\,mg\,kg^{-1}$, but pesticide application and waste disposal can result in much higher concentrations (WHO, 2001).

General population exposure to arsenic is mainly via intake of food and drinking water. Food is the most important source, but in some areas arsenic in drinking water is a significant source of exposure to inorganic arsenic. Contaminated soils such as mine-tailings are also a potential source of arsenic exposure (WHO, 2001). Absorption of arsenic in inhaled airborne particles is highly dependent on the solubility and the size of particles. Soluble arsenic compounds are easily absorbed from the gastrointestinal tract. However, inorganic arsenic is extensively methylated in humans and the metabolites are excreted in the urine (WHO, 2001).

Arsenic (or metabolites) concentrations in blood, hair, nails, and urine have been used as biomarkers of exposure. Speciated metabolites in urine expressed as either inorganic arsenic or the sum of metabolites (inorganic arsenic + MMA + DMA) is generally the best estimate of recent arsenic dose. However, consumption of certain seafoods may confound estimation of inorganic arsenic exposure, and should therefore be avoided before urine sampling (WHO, 2001).

12.6.2   Health effects

Inorganic arsenic is acutely toxic and intake of large quantities leads to gastrointestinal symptoms, severe disturbances of the cardiovascular and central nervous systems, and eventually death. In survivors, bone marrow depression, haemolysis, hepatomegaly, melanosis, polyneuropathy, and encephalopathy may be observed. Ingestion of inorganic arsenic may induce peripheral vascular disease, which in its extreme form leads to gangrenous changes (black foot disease, only reported in Taiwan) (Järup, 2003).

Populations exposed to arsenic via drinking water show excess risk of mortality from lung, bladder, and kidney cancer, the risk increasing with increasing exposure. There is also an increased risk of skin cancer and other skin lesions, such as hyperkeratosis and pigmentation changes. Studies of various populations exposed to arsenic by inhalation, such as smelter workers, pesticide manufacturers, and miners in many different countries, consistently demonstrate an excess lung cancer. Although all these groups are exposed to other chemicals in addition to arsenic, there is no other common factor that could explain the findings. The lung cancer risk increases with increasing arsenic exposure in all relevant studies, and confounding by smoking does not explain the findings (Järup, 2003).

A WHO (2001) evaluation concludes that arsenic exposure via drinking water is causally related to cancer in the lungs, kidney, bladder, and skin, the last of which is preceded by directly observable precancerous lesions. Uncertainties in the estimation of past exposures are important when assessing the exposure–response relationships, but it would seem that drinking water arsenic concentrations of approximately $100\,\mu g\,L^{-1}$ have led to cancer at these sites, and that precursors of skin cancer have been associated with levels of 50–$100\,\mu g\,L^{-1}$. The relationships between arsenic exposure and other health effects are less clear. There is relatively strong evidence for hypertension and cardiovascular disease, but the evidence is only suggestive for diabetes and reproductive effects and weak for cerebrovascular disease, long-term neurological effects, and cancer at sites other than lung, bladder, kidney, and skin (WHO, 2001).

Millions of people are at risk of cancer and other diseases because of chronic arsenic exposure (Flora *et al.*, 2007). General adverse health effects associated with human exposure to arsenicals include cardiovascular diseases, developmental abnormalities, neurologic and neurobehavioural disorders, diabetes, fibrosis of the liver and lung, and hematological disorders (Tseng, 2007). Once in the tissue, arsenic exerts its toxic effects through several mechanisms, the most significant of which is the reversible combination with sulfydryl groups. No suitable molecular markers of arsenic toxicity are available at present. Blood/urine concentration

of arsenic is currently applied to determine the body/tissue burden of arsenic. Several workers have estimated arsenic concentration in toenails of humans suffering from arseniasis. These observations have been generally considered as biomarkers of exposure (Yoon *et al.*, 2008).

12.7 Nickel

12.7.1 Occurrence, use and exposure

Nickel (Ni) is ubiquitous in nature. Small amounts of Ni are needed by the human body to produce red blood cells; in excessive amounts, it can however become mildly toxic. It occurs mainly in the form of sulfide and silicate minerals. Ambient air, as a result of industrial activity, combustion of fossil fuels, and waste incineration, is known to contain very low levels of nickel. Human exposure may occur through inhalation, ingestion, and dermal contact. Occupational exposure may be caused by elemental nickel, nickel compounds, complexes, and alloy, and also the fumes from alloys used in welding and brazing (Yoon *et al.*, 2008).

Food is a major route of exposure for many people. The Environmental Protection Agency estimates that an average adult consumes 100–300 μg of nickel per day. Drinking water contains very small amounts of nickel (ATSDR, 1997). Deposition, absorption, and elimination of nickel particles in the respiratory tract largely depend upon the particle size and concentration of nickel. The rate of dermal absorption depends on the rate of penetration in the epidermis, which differs for different forms of nickel. When administered to animals, nickel is rapidly distributed to the kidneys, pituitary, lungs, skin, adrenals, ovaries, and testes (Sunderman, 1989). Although intracellular ligands for Ni have not been fully characterized, Sunderman (1989) suggested that cysteine, histidine, and aspartic acid may form nickel complexes. It is poor inducer of metallothionein. A nickel binding metalloproteinnickeloplasmin has been identified in plasma. It is an α-1 glycoprotein complex that plays an important role in extracellular transport, intracellular

binding, and urinary and biliary excretion of nickel (Nebor and Hriagu, 1992; Tabata and Sarkar, 1992). Human nutritional requirements for nickel have not been established (WHO, 1996).

Nickel compounds are carcinogenic to human (IARC, 1989). Risks were highest for lung and nasal cancers among workers heavily exposed to nickel sulfide, nickel oxide, and metallic nickel.

12.7.2 Health effects

It has been hypothesized that nickel damages DNA directly through reactive oxygen species (McCoy and Kenney, 1992). This hypothesis is supported by the fact that the antioxidant vitamin E inhibits some chromosomal condensation caused by nickel (Lin *et al.*, 1991). Blood nickel levels provide a guideline as to the severity of exposure. Sodium diethyldithiocarbamate provides protection against clinical effects.

12.8 Other essential elements

There is a group of eight metals generally accepted as essential: cobalt, copper, iron, magnesium, manganese, molybdenum, selenium, and zinc. A brief account on the 'toxicity' of these metals deserves a separate mention.

12.8.1 Copper

Copper (Cu) is a nutritionally essential element to human life, but in high doses it can cause anemia, liver and kidney damage, and stomach and intestinal irritation. In a general population, food, beverages, and drinking water are potential sources of excess exposure. Industrial exposure occurs in miners or through smelting operations, welding and related activities (Yoon *et al.*, 2008). The metabolism and health effects of copper have been reviewed by WHO (1996), Chan *et al.* (1998), NRC (2000), and others. Gastrointestinal absorption of copper is normally regulated by homeostatic mechanisms. It is transported through serum initially bound to albumin and later more firmly to ceruloplasmin and transcuprein.

The bile is the normal excretory pathway that plays a primary role in copper homeostasis.

Copper toxicity occurs in the form of nausea, vomiting and diarrhea (Pizzarro *et al.*, 1999). Ingestion of large amounts of copper salts may produce hepatic necrosis and death. Excessive accumulation of copper in the liver, brain, kidneys, and cornea manifests into Wilson's disease. This disorder is also referred to as hepatolenticular degeneration. Genetic studies have identified a linkage between Wilson's disease and chromosome 13. Menke's disease or 'Kinky hair syndrome', Indian childhood cirrhosis (ICC), non-Indian childhood cirrhosis, and idiopathic copper toxicosis are other disorders caused by copper (Mercer *et al.*, 1993; Sethi *et al.*, 1993; Muller *et al.*, 1996).

12.8.2   Selenium

Selenium (Sn) is needed by humans and other animals in small amounts, but in larger amounts can cause damage to the nervous system, fatigue, and irritability. Selenium accumulates in living tissue, causing high selenium content in fish and other organisms, and causing greater health problems in a human over a lifetime of overexposure. These health problems include hair and fingernail loss, damage to kidney and liver tissue, damage to circulatory tissue, and more severe damage to the nervous system (Yoon *et al.*, 2008).

Selenium metabolism is regulated to meet several metabolic needs. The requirement for selenium is related to the degree of oxidant activity and the supply of nutrients such as zinc, copper, manganese, iron, and vitamin E. Selenium deficiency leads to cardiomyopathy in mammals, including humans (Levander and Burk, 1996). Keshan disease is caused in humans due to selenium deficiency. This is an endemic cardiomyopathy first discovered in Keshan county in the People's Republic of China in 1935 (Chen *et al.*, 1980). In livestock and horses, its toxicity is recognized as 'alkali disease' characterized by loss of vitality, emaciation, deformity, and shedding of hoofs, loss of long hair, and erosion of joints of long bones. Deficiency of vitamin E increases selenium toxicity. It protects cadmium toxicity.

12.8.3   Manganese

Manganese (Mn) is an essential element. It can exist in 11 oxidation states, from –3 to +7. The most common valences are +2, +4, and +7. In superoxide dismutase, it exists during oxidative stress. It is a cofactor for a number of enzymatic reactions, particularly those involved in phosphorylation, cholesterol, and fatty acid synthesis. Manganese concentrates in mitochondria so the tissues rich in these organelles including liver, pancreas, kidneys, and intestine have the highest concentration of manganese. It is eliminated through bile and is reabsorbed by the intestine, but the principal route of excretion is feces. The most common form of manganese toxicity occurs through chronic inhalation of airborne manganese in miners, steel mills, and some chemical industries (ATSDR, 1997). Pathologic changes include epithelial necrosis followed by mononuclear proliferation. Chronic manganese toxicity is called as 'Manganism'. It is a neuropsychiatic disorder characterized by irritability, difficulty in walking, speech disturbances, and compulsive behavior that may include running, fighting, and singing (Yoon *et al.*, 2008).

12.8.4   Molybdenum

Molybdenum (Mo) exists in multiple oxidation states +3, +4, +5, and +6, facilitating electron transfer. In humans, chronic exposure to molybdenum is characterized by high uric acid level in serum and urine. It is referred to as molybdenosis. In India, it is known as 'genu valgum'. Gout-like disease has been observed in inhabitants of a province of former USSR (Chan *et al.*, 1998). After repeated oral administration in rats, fatty degeneration of liver and kidney was observed (Nielson, 1996). Cotter and Gunsalus (1989) showed that molybdenum was required both for complete induction of dmsAlacZ expression during anaerobic growth of *Escherichia coli*. Kolesnikow *et al.* (1992) provided the first evidence that NarL and FNR interact to ensure optional expression of nrK gene. The availability of molybdate and iron is necessary for optimal nrK expression, whereas the availability of nitrate is not essential.

12.8.5 Zinc

Zinc (Zn) is a nutritionally essential metal and a deficiency results in severe health consequences. Excessive exposure to zinc is relatively uncommon and occurs only at very high levels. Seafood, meat, whole grains, dairy products, nuts, and legumes are high in zinc (Yoon *et al.*, 2008). More than 200 metalloenzymes belonging to six major categories (namely oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases) require zinc as a cofactor (Cousins, 1996). Zinc induces the synthesis of metallothioneins which is a factor in regulating the metabolism of zinc including absorption and storage (Miles *et al.*, 2000). Zinc is a functional component of several proteins that contribute to gene expression and regulation of genetic activity. Zinc chelates with cysteine and/or histidine in a tetrahedral configuration forming looped structures called 'zinc fingers' which bind to specific DNA regions and are bound in various transcription factors such as steroid hormone receptors and polymerase (Wang *et al.*, 1997). It has a role in immune function and the cytokines.

Inhalation of freshly formed fumes of zinc has been associated with metal fume fever. While the mechanisms of zinc ions interaction with immune cells are poorly understood, a striking concurrent effect of zinc is the induction of the biosynthesis of metallothionein (MT), a group of low-molecular-weight cysteine-rich metal binding proteins, believed to play a role in zinc homeostasis (Yoon *et al.*, 2008).

12.8.6 Cobalt

Cobalt (Co) is a naturally occurring element that has properties similar to those of iron and nickel. Cobalt has both beneficial and harmful effects to human health. Cobalt is beneficial for humans because it is part of vitamin B12, which is essential to maintain human health. Cobalt (0.16–1.0 mg kg^{-1} of body weight) has also been used as a treatment for anemia, including in pregnant women, because it causes red blood cells to be produced. Cobalt also increases red blood cell production in healthy people, but only at very high exposure levels. Cobalt is also essential for the health of various animals, such as cattle and sheep. Exposure of humans and animals to levels of cobalt normally found in the environment is not harmful (ATSDR, 2004).

Cobalt may enter the environment from both natural sources and human activities. Plants can accumulate very small amounts of cobalt from soil, especially in the parts of the plant that we eat most often such as the fruit, grain, and seeds. Once cobalt enters the body, it is distributed into all tissues but mainly into the liver, kidney, and bones. After cobalt is breathed in or eaten, some of it leaves the body quickly in the feces. The rest is absorbed into the blood and then into the tissues throughout the body. The absorbed cobalt leaves the body slowly, mainly in the urine (ATSDR, 2004).

When too much cobalt is taken into your body, however, harmful health effects can occur. Workers who breathed air containing 0.038 mg m^{-3} cobalt (about 100,000 times the concentration normally found in ambient air) for 6 hours had trouble breathing. Serious effects on the lungs, including asthma, pneumonia, and wheezing, have been found in people exposed to 0.005 mg m^{-3} cobalt while working with hard metal, a cobalt-tungsten carbide alloy. People exposed to 0.007 mg m^{-3} cobalt at work have also developed allergies to cobalt that resulted in asthma and skin rashes. The general public, however, is not likely to be exposed to the same type or amount of cobalt dust that caused these effects in workers (ATSDR, 2004).

In the 1960s, some breweries added cobalt salts to beer to stabilize the foam (resulting in exposures of 0.04–0.14 mg kg^{-1} cobalt). Some people who drank excessive amounts of beer (8–25 pints/day) experienced serious effects on the heart. In some cases, these effects resulted in death. Nausea and vomiting were usually reported before the effects on the heart were noticed (ATSDR, 2004).

12.8.7 Iron

Iron (Fe) is the most abundant trace mineral in the body and is an essential element in most biological systems. It is likely that iron was essential for developing aerobic life on Earth (Williams,

1990). About 70% of the iron in mammals is found in hemoglobin, and about 5% to 10% is found in myoglobin. When bound to normal hemoglobin and myoglobin, iron is in the ferrous (Fe^{2+}) form. Up to 25% of iron in the body is in the ferric (Fe^{3+}) form and is stored in hemosiderin, ferritin, and transferring in the liver, spleen, and bone marrow. Ferric iron is used in iron-containing enzymes such as peroxidase, catalase, and cytochrome (Goyer, 1996).

Iron is toxic to cells in excessive amounts, however. Acute iron poisoning is common and potentially lethal in dogs, cats, and many other animals. Iron is also a leading cause of unintentional poisoning deaths in children less than 6 years old. Since no mechanism exists for excreting iron, toxicity depends on the amount of iron already in the body. Consequently, some animals develop clinical signs of toxicosis even when they receive doses that cause no problems in other animals. Iron is most toxic when given intravenously. Intramuscular injections are less toxic and iron given orally is the least toxic, probably because the amount of iron absorbed orally is not 100% of the dose ingested. When assessing the potential toxicity of an iron overdose, the amount of elemental iron in the products ingested must be determined (Greentree and Hall, 1995).

12.8.8   Magnesium

Magnesium (Mg) is the fourth-most abundant mineral in the body and is essential to good health. Approximately 50% of total body magnesium is found in bone. The other half is found predominantly inside cells of body tissues and organs. Only 1% of magnesium is found in blood, but the body works very hard to keep blood levels of magnesium constant (USDA, 2011). Magnesium is needed for more than 300 biochemical reactions in the body. It helps maintain normal muscle and nerve function, keeps heart rhythm steady, supports a healthy immune system, and keeps bones strong. Magnesium also helps regulate blood sugar levels, promotes normal blood pressure, and is known to be involved in energy metabolism and protein synthesis. There is an increased interest in the role of magnesium in preventing and managing disorders such as hypertension, cardiovascular disease, and diabetes. Dietary magnesium is absorbed in the small intestines. Magnesium is excreted through the kidneys (Ford and Mokdad, 2003).

Dietary magnesium does not pose a health risk; however, pharmacologic doses of magnesium in supplements can promote adverse effects such as diarrhea and abdominal cramping. Risk of magnesium toxicity increases with kidney failure, when the kidney loses the ability to remove excess magnesium. Very large doses of magnesium-containing laxatives and antacids also have been associated with magnesium toxicity (Xing and Soffer, 2001). Signs of excess magnesium can be similar to magnesium deficiency and include changes in mental status, nausea, diarrhea, appetite loss, muscle weakness, difficulty breathing, extremely low blood pressure, and irregular heartbeat (Jaing et al., 2002).

12.9   Conclusions

Heavy metals are toxic to human health and they also represent a threat to the environment due to their tendency to accumulate in different environmental components. However, some metals at trace levels are essential for various biological processes in the human body. Infants and children are particularly susceptible to neurotoxicological damage from metal exposure due to their ongoing rapid intellectual development. The neurological systems of fetuses, often the target of damage from metals, are also at risk because of the transfer of maternal blood in which metals may bioaccumulate. These compounds should therefore be subject to mandatory monitoring and management. Governments should promote harmonized data collection, research, legislation, and regulations, and consider the use of indicators. When setting acceptable levels or criteria related to chemicals, the potential enhanced exposures and/or vulnerabilities of children should be taken into consideration.

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13

Monitoring and Health Risk Assessment of Heavy Metal Contamination in Food

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Summary

Contamination of food with heavy metals not only affects the nutritive values of these foods but also affects the health of human beings; safe limits of these heavy metals are therefore lowered regularly in foodstuffs. This regulation is the responsibility of national and international regulatory authorities. A health risk assessment of heavy metals by the population is a very good technique, as it could provide information about any threat regarding heavy metals contamination in food commodities.

13.1 Introduction

Food safety is a major public concern worldwide. The increasing demands for food and food safety has drawn the attention of researchers to the risks associated with consumption of contaminated foodstuffs i.e. pesticides, heavy metals, and/or toxins in vegetables (DMello, 2003). Contamination of foods by heavy metals has become an inevitable challenge these days. Air, soil, and water pollution are contributing to the presence of harmful elements, such as cadmium, lead, and mercury in foodstuff. The occurrences of heavy-metals-enriched ecosystem components arise from rapid industrial growth, advances in agricultural chemicalization, or the urban activities of human beings. These agents have led to metal dispersion in the environment and, consequently, impaired health of the population by the ingestion of victuals contaminated by harmful elements (Zukowska and Biziuk, 2008).

Flooding from heavy downpour may lead to horizontal leaching from dump sites causing metal uptake by roots of crops; the rest may find their way into open water bodies and the entire aquatic ecosystem. The entry into the food chain of these metals leads to increased susceptibility and exposure to metal poisoning of the local population (Arora *et al.*, 2008).

Among other routes, food is one of the main sources of consumer exposure to heavy metals. Since increased dietary metals intake may contribute to the development of various disorders, there is a necessity to monitor these substances in the human diet. Heavy metals show a significant build-up with contamination, and long-term accumulation of heavy metals in soils has led to contamination of food crops. It is recommended that people living in such areas should not eat large quantities of these foods, in order to avoid excessive accumulation of heavy metals in the body. Dietary intake of food results in long-term low-level body accumulation of heavy metals, and the detrimental impact only becomes apparent after several years of exposure. Regular monitoring of these toxic heavy metals from effluents and sewage in foods is essential, to prevent their excessive build-up in the food chain (Orisakwe *et al.*, 2012).

A number of serious health problems can develop as a result of excessive uptake of dietary heavy metals. Furthermore, the consumption of heavy-metal-contaminated food can seriously deplete essential nutrients in the body, causing a decrease in immunological defenses, intrauterine growth retardation, impaired psycho-social behaviors, disabilities associated with malnutrition, and a high prevalence of upper gastrointestinal cancer (Arora *et al.*, 2008).

In this respect, different techniques and methodologies of food analysis for heavy metal contaminants, as well as risk assessment associated with dietary intakes of heavy metals, will be presented.

13.2   Analytical methods

There are two basic types of analytical methods for assaying heavy metals: colorimetric and instrumental methods. The classical methods are colorimetric, where the concentrations of heavy metals are measured as a group of like elements. The newer instrumental methods measure individual elements.

13.2.1   Colorimetric methods

Colorimetric analytical methods (AHPA, 2009) have been in use for over 100 years and are based on measuring color changes of solutions that arise from specific chemical interactions. The current test creates a chemical reaction that is compared with a standard prepared from stock lead nitrate. It relies on the ability of lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper, and molybdenum to react with thioacetamide-glycerin base test solution (TS) at a pH of 3.5 to produce a color that is then compared with the standard preparation. It can be used to demonstrate that the content of metallic impurities colored by sulfide ions under the specific test conditions do not exceed a certain limit.

In order to prepare botanical and herbal dietary supplement samples for colorimetric analysis, they must undergo a chemical reaction that, depending on the method, requires a decarbonization step with concentrated nitric and sulfuric acids followed by digestion with hydrochloric acid, or digestion with concentrated nitric and sulfuric acids followed by hydrogen peroxide if needed. The advantage of this method is that it can be performed using basic glassware and normal laboratory reagents and equipment. It does not require any expensive instrumentation. The disadvantages, however, are that the detection limit for colorimetric methods is within the range 10–20 ppm where all the responding metals, including some beneficial elements such as copper, molybdenum, tin, and silver, are also measured as lead equivalents. The use of this method therefore cannot ensure that heavy metal specifications established at very low levels are met. Additionally, heavy metals are not well recovered by this method and mercury is not recovered at all. Another colorimetric test is a procedure for measuring lead by selectively extracting it from the sample. This procedure is fairly long and uses sulfuric acid, hydrogen peroxide, potassium cyanide, dithizone, and chloroform.

13.2.2 Instrumental methods

There are four instrumental methods (AHPA, 2009) routinely used to measure heavy metal levels. They are flame atomic absorption spectroscopy (FAAS), graphite furnace atomic absorbance spectroscopy (GFAAS), inductively coupled plasma atomic emission spectroscopy (ICP-AES), and inductively coupled plasma mass spectroscopy (ICP-MS).

The sample preparation for all these methods relies on digestion of the sample using concentrated nitric acid and/or hydrochloric acid and hydrogen peroxide. FAAS is the oldest of these techniques and relies upon the electrochemical properties of metals that allow them to absorb energy from light of specific wavelengths. The greater the number of atoms of a selected element that are exposed to the correct wavelength and absorb it, the greater the total amount of light absorbed. The relationship between the amount of light absorbed and the concentration of analysts present in known standards can be used to determine sample concentrations by measuring the amount of light that they absorb.

GFAAS is similar to FAAS, but uses a different sampling system. FAAS uses a relatively inefficient system where only a small fraction of the sample reaches the atomizing flame before quickly passing through the light path. GFAAS uses an improved sampling device that atomizes the entire sample and retains it in the light path for an extended period of time. This is done by replacing the flame used in FAAS with an electrically heated graphite tube. These changes significantly improve the detection limits of the technique.

ICP-AES uses argon inductively coupled plasma maintained by the interaction of a radio frequency field and ionized argon gas to excite atoms to unstable energy configurations. The excess atomic energy is released as emitted light when the atoms return to more stable configurations. The wavelengths of the energy released are specific to the elements in the sample, and the intensity of the emission is a function of the concentration of atoms that are affected. ICP temperatures reach as high as 10,000 K with samples experiencing temperatures between 5500 and 8000 K. ICP-MS retains the

sample introduction system used in ICP-AES but the atomic ions produced by the argon plasma are directed into a mass spectrometer (MS). The MS separates the ions introduced from the ICP according to their mass-to-charge ratio. Ions of the selected mass-to-charge ratio are directed to the detector, which records the ions present. This provides identification and quantification of the elements of interest.

Typically a quadruple mass analyzer spectrometer is used due to its ease of use, robustness, and speed. However, other mass analyzer systems such as ion-trap, sector field, and time of flight can be used.

A fifth instrumental method, x-ray fluorescence spectrometry (XRF), is seeing some use as a screening tool due to the availability of hand-held field instruments. XRF employs x-rays to ionize elements and records the characteristic emissions of atoms as they return to more stable energy states. It is fast, relatively inexpensive, requires minimal sample preparation, can identify many elements at once, but is only moderately sensitive (AHPA, 2009).

13.3 Contamination levels data

In this section we present data exemplified concentration levels of heavy metals in different types of food commodities, categorized according to the foodstuff item.

13.3.1 Vegetables and fruits

A market basket survey was carried out with the aim of assessing the levels of lead (Pb), cadmium (Cd), copper (Cu) and zinc (Zn) in various fruits and vegetables sold in Egyptian markets (Radwan and Salama, 2006). The results of this survey showed that the average concentrations detected were within the ranges 0.01–0.87, 0.01–0.15, 0.83–18.3 and 1.36–20.9 mg kg^{-1} for Pb, Cd, Cu, and Zn, respectively. The highest mean levels of Pb, Cd, Cu, and Zn were detected in strawberries, cucumber, date, and spinach, respectively. The daily intakes of Pb, Cd, Cu, and Zn through fruits and vegetables

were found to be below the recommended tolerable levels proposed by Joint FAO/WHO Expert Committee on Food Additives 1999 and may not constitute a health hazard for consumers.

Tufuor *et al.* (2011) determined As, Pb, Cr, Ni, Cu, Zn, and Fe in oranges, limes and lemons collected from different farms in the Abura-Asebu–Kwamankese District of the Central Region of Ghana. The metals detected were found to be highest in the lime juice, probably due to its high acidity and sequestration ability. These fruits contain acids such as citric, tartaric, and malic acids that have the power of chelating heavy metals (Tufuor *et al.* 2011). The chelating power partly depends on the acidity of the fruit and, since the acidity of the three citrus fruits has been found to be in the order lime > lemon > orange (Braverman 1949; Chulme 1981), it is therefore not surprising that lime had the highest concentration of trace metals. The mean concentrations of the trace metals were found to be in the order: As < Cu < Pb < Zn < Fe. The authors reported that the levels of all the metals were far lower than dietary reference values recommended by the United Kingdom Department of Health and the US EPA.

Arora *et al.* (2008) determined different heavy metals such as iron, manganese, copper, and zinc in vegetables irrigated with water from different sources in India. The results indicated a substantial build-up of heavy metals in vegetables irrigated with wastewater. The measured metals showed differential accumulation in the analyzed vegetables. The range of various metals in wastewater-irrigated plants was 116–378, 12–69, 5.2–16.8, and 22–46 mg kg^{-1} for iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn), respectively. The highest mean level of Fe was detected in mint and the lowest in radish, whereas the level of Zn was highest in carrot and lowest in radish and brinjal (Figure 13.1). The study revealed that both adults and children consuming vegetables grown in wastewater-irrigated soils ingest significant amounts of these metals. However, the values of these metals were below the recommended maximum tolerable levels.

Yang *et al.* (2011) monitored concentration levels of some heavy metals (e.g. Pb, Zn, Mn, Cu,
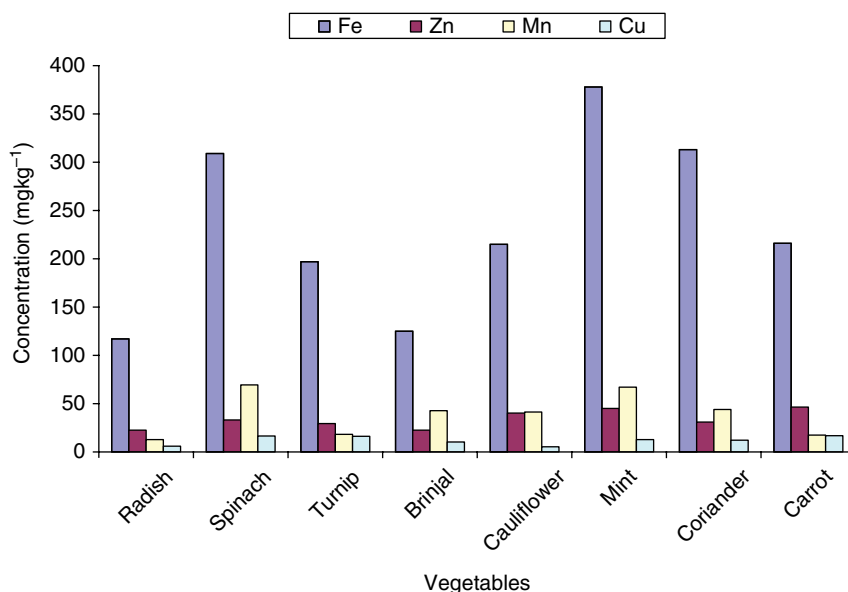
Figure 13.1   Heavy metal content (mg kg^{-1} dry weight) in plants grown in wastewater-irrigated soils. Scientific names of plants: radish: *Raphanus sativus*; spinach: *Spinacia oleracea*; turnip: *Brassica rapa*; brinjal: *Solanum melogena*; cauliflower: *Brassica oleracea* var. botrytis; mint: *Mentha requienii*; coriander: *Coriandrum sativum*; carrot: *Daucus carota*. Adapted from Arora *et al.* (2008). For color details, see color plates section

Cd, and Cr) in market vegetables in Chongqing, China and estimated their potential health risk for local consumers. The results showed that the measured Pb and Cd concentrations exceeded the safety limits given by FAO/WHO and Chinese regulations, indicating serious contamination of market vegetables by these metals. Based on the author's results, it appears that the highest concentration of Pb based on dry weights (14.9 mg kg^{-1}) was detected in mustard followed by spinach (12.8 mg kg^{-1}). Garlic leaf retained higher Pb (10.4 mg kg^{-1}) than the stem (9.72 mg kg^{-1}). A similar trend was observed for Pb in leaf and stem of asparagus (Figure 13.2). Contamination levels for Zn and Cd were also higher in the leaves than in the stems of both plants. The opposite was obtained for Cu in leaf and stem of both garlic and asparagus, where the stem contained higher Cu than the leaf (Figure 13.2).

13.3.2   Medicinal plants and herbs

Subramanian *et al.* (2012) determined the heavy metals lead (Pb), zinc (Zn), cadmium (Cd), and copper (Cu) in 15 different medicinal plants

regularly used in cooking in Indian curries. The average concentrations detected fell within the ranges 0.478–9.890, 6.94–49.76, 0.684–2.751, and 11.51–94.05 mg kg^{-1}, respectively, for the above-mentioned metals. The mean heavy metal concentrations were found to be below the recommended maximum acceptable levels proposed by the Joint FAO/WHO Expert Committee on Food Additives.

Heavy metals (e.g., Fe, Cu, Mn, Zn, Pb, Co, Cr, Cd, Sn, Ni) were detected at different levels in some medicinal plants, such as peppermint (*Mentha piperita* L.), chamomile flowers (*Matricaria chamomilla* L.), caraway (*Carum carvi* L.), tilio (*Lindin blossom* L.), and anise (*Pimpinella anisum* L.). According to Abou-Arab *et al.* (1999), the highest mean levels of Pb, Zn, Cu, and Fe were found in chamomile flowers, those of Cd, Cr, and Mn in peppermint, and those of Ni, Co, and Sn in caraway samples (Table 13.1). If the levels set up by the 1986 ZEBS regulation (Schilcher *et al.*, 1987) for lead (0.25 mg kg^{-1}) and cadmium (0.1 mg kg^{-1}) in fruits or vegetables are considered, it can be observed that only chamomile samples had a lead concentration (0.31 mg kg^{-1}) higher than the recommended limits. In contrast, all the analyzed samples contained cadmium at levels higher than the recommended limit.
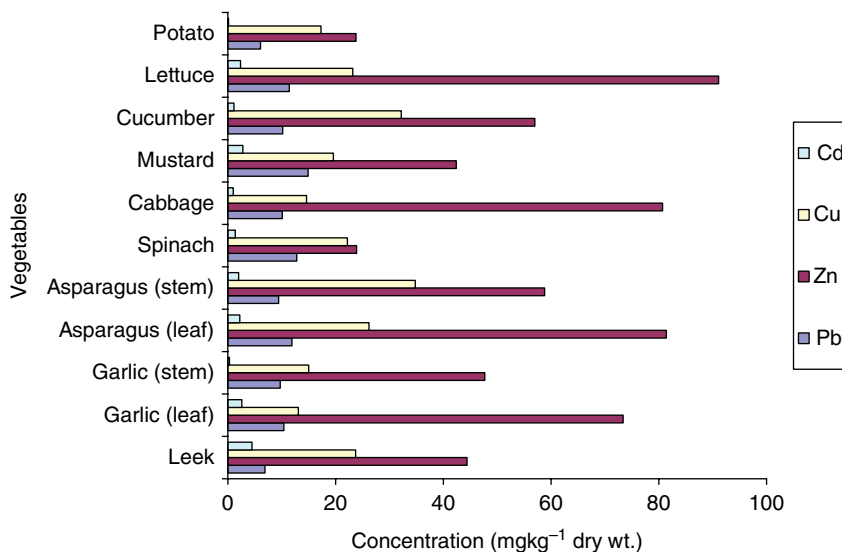

Figure 13.2   Mean concentrations (mg kg^{-1} dry weight) of heavy metals in some vegetables sampled from Chongqing markets, China. Adapted from Yang *et al.* (2011). For color details, see color plates section

Table 13.1   Concentration levels (mg kg^{-1}) of heavy metals in medicinal plants collected from Egyptian markets (ND: not detected). Source: Abou-Arab *et al.*, 1999. Reproduced with permission of Elsevier.

Metal	Peppermint	Chamomile	Anise	Caraway	Tilio
Pb	0.081	0.308	0.224	0.214	0.121
Cd	0.306	0.211	0.081	0.226	0.141
Cr	0.096	0.069	0.088	0.050	0.096
Ni	ND	ND	0.106	0.814	ND
Co	ND	ND	0.042	0.162	0.064
Zn	38.8	122.0	61.8	38.0	60.4
Mn	38.8	28.8	36.6	28.4	36.4
Cu	6.90	10.40	4.10	1.38	4.90
Fe	84.8	125.0	36.6	66.4	49.4
Sn	ND	ND	ND	0.040	ND

Obi *et al.* (2006) characterized the content of cadmium, copper, iron, nickel, selenium, zinc, lead, and mercury in a total of 25 random samples of Nigerian traditional products. The result showed that 100% of the samples contained elevated amounts of heavy metals. These data alert us to the possibility of heavy metal toxicity from herbal products in Nigeria. The authors added that the public health hazards from ingestion of herbal medicines should be identified and disclosed by in-depth risk assessment studies.

13.3.3   Grains

The most important crop worldwide in basic food commodities is wheat (*Triticum aestivum* L.), followed by coarse grains and rice, with a global production of 626 million tons in 2007 (FAO, 2007) The average worldwide per-capita consumption in 2005 was 68 kg, with 61 kg and 95 kg being consumed in developing and developed countries, respectively (Bermudez *et al.*, 2011).

Fu *et al.* (2008) determined concentrations of 10 heavy metals (As, Ba, Cd, Co, Cr, Cu, Hg, Mn, Ni, and Pb) in 13 polished rice and relevant hull samples, as well as six relevant paddy soil samples. The geometric mean concentrations of Cd, Cu, and Hg in soil samples were 4.0, 2.0 and 1.1, respectively, times the maximum allowable concentration (MAC) set for Chinese agricultural soils. The analyzed metal concentrations were significantly different between rice and relevant

hull except for As, Cd and Hg ($p < 0.05$). All metal concentrations in rice hull (except for Co) were higher than those in polished rice. The geometric mean of Pb in polished rice was 3.5 times that of the MAC for milled rice. Cd contents in 31% of the rice samples exceeded the national MAC, and the arithmetic mean also slightly exceeded national MAC. In addition, Cd and Pb contents in local rice were much higher than commercial rice samples examined in this work and previous studies. Comparing the tolerable daily intakes given by FAO/WHO with the mean estimated daily intakes, the authors found that Pb daily intake through rice consumption in this area exceeded the FAO tolerable daily intake, and the Cd daily intake comprised 70% of the total tolerable daily intake. The daily intake of Hg and As through rice was much lower than the tolerable daily intakes, but bioaccumulation of Hg through the food chain and intake of As from other food stuff should also be of concern.

Cao *et al.* (2010) investigated heavy metal (Cu, Zn, Pb, Cr, Hg, and Cd) concentrations in rice and garden vegetables, as well as in cultivated soils, in a rural industrial-developed region in southern Jiangsu, China and estimated the potential health risks of metals to the inhabitants via consumption of locally produced rice and garden vegetables. Average concentrations of Cr, Cu, Zn, Cd, Hg, and Pb were 0.75, 2.64, 12.00, 0.014, 0.006, and 0.054 mg kg^{-1} dw (dry weight) in rice and were 0.67, 1.18, 4.34, 0.011, 0.002, and

0.058 mg kg⁻¹ fw (fresh weight) in garden vegetables, respectively. These values were all below the maximum allowable concentration in food in China except for Cr in vegetables. Leafy vegetables had higher metal concentrations than Solanaceae vegetables. Average daily intake of Cr, Cu, Zn, Cd, Hg, and Pb through the consumption of rice and garden vegetables were 5.66, 16.90, 74.21, 0.10, 0.04, and 0.43 µg kg⁻¹ day⁻¹, respectively. Although hazard quotient values of individual metals were all lower than 1, when all six metal intakes via self-planted rice and garden vegetables were combined, the hazard index value was close to 1. Potential health risks from exposure to heavy metals in self-planted rice and garden vegetables need more attention.

The FAO European Co-operative Research Network on Trace Elements reported the results of heavy metals (lead, cadmium, molybdenum, and nickel) and nutrients with potential toxicity (selenium, copper, zinc, and manganese) in products from the cereals group, being part of total diet in Madrid. Pasta showed the highest levels for cadmium (50.4±0.4 µg kg⁻¹ dw), molybdenum (308±6 µg kg⁻¹ dw), nickel (383±5 µg kg⁻¹ dw), selenium (110±3 µg kg⁻¹ dw), and copper (3.81±0.09 mg kg⁻¹ dw); whole bread, the highest of lead (59.2±8.0 µg kg⁻¹ dw), zinc (18.4±0.1 mg kg⁻¹ dw), and manganese (17.1±0.3 mg kg⁻¹ dw). The total dietary intake was estimated for each element and compared with relevant international standards such as the Provisional Tolerable Weekly Intake (PTWI) (FAO/WHO, 1993) and Reference Doses (RfD). The dietary intake of all elements from the cereal group contributes less than 15% of the maximum permissible international intake standards (Cuadrado et al., 2000).

Heavy metal and trace element concentrations were examined in wheat grains and straw to elucidate associations between air pollution sources and soil variables (Bermudez et al., 2011). The sampling zone included seven areas of Cordoba province, located in central Argentina. A total of 34 sampling points were chosen in seven areas sampled before the wheat harvest. The mean wheat grain concentrations of Cr, Cu, Fe, Mn, and Zn surpassed the tolerance limits stated in the international legislation for wheat grain and foodstuffs. When topsoil Ba, Co, Cr, and Zn concentrations were higher than the legislation thresholds for agricultural and residential soils, wheat grain concentrations were also increased. In addition, Cr, Cu, Mn, Ni, Pb, and Zn revealed an immobilization effect of a cement plant and the atmospheric deposition input, with Cd in wheat grains being associated with a cement plant and industrial waste incinerator. The health risks arising from wheat grain consumption indicated that the inhabitants of Argentina are experiencing significant non-carcinogenic risks (Hazard Index=3.311), especially when consuming wheat grains affected by metallurgical or chemical factories as well as by air transportation from big cities.

13.3.4 Fish and seafood

The contents of Cd, Cu, Cr, Hg, Ni, and Zn in muscle, liver, and gills were studied in whitefish, perch, pike, brown trout, burbot, and vendace from three lake localities in a watercourse in the border region between Norway and Russia, in the vicinity of mining activity and several metallurgic smelters (Amundsen et al., 1997). The contents of Cd and Ni in fish tissue increased with increasing proximity to the smelters, whereas the other elements showed similar concentrations at the three localities. The recorded heavy metal concentrations appeared to be within the ranges reported for fish from other metal-contaminated lakes, and higher than comparable observations from unpolluted systems. The heavy metal concentrations were usually lowest in muscle and highest in the liver or the gills. Significant differences in metal concentration levels were found between different fish species, but Hg was the only metal where these species differences were possibly related to biomagnification. For the other elements, the concentrations generally appeared to be inversely related to the trophic level of the fish species.

The fish samples rahu (Labeo rohita), tilapia (Tilapia zilli) and catfish (Chrysichthys nigrodigatatus) were collected from Yamuna River in Delhi for determination of Al (aluminum), B (boron), Ba (barium), Cd (cadmium), Co (cobalt),

Cr (chromium), Cu (copper), Fe (iron), K (potassium), Mg (magnesium), Mn (manganese), Na (sodium), Ni (nickel), Pb (lead), Sb (antimony), Sn (tin), Si (silicon), P (phosphorus) and Zn (zinc) using the inductively coupled plasma optical emission spectroscopy (ICP-OES) technique (Sen *et al.*, 2011). The concentrations of Ca, K, Mg, Na, and P were found too high as compared with other metals which were below the maximum permissible level set by the World Health Organization (WHO). Si, Pb, and Fe also showed high concentration values in the fish samples. This pollution may be a result of local industries or from drainage lines which are connected to the river Yamuna.

13.3.5   Miscellaneous

Roychowdhury *et al.* (2003) determined heavy metals in food composites from the Jalangi block, Murshidabad district and Domkal block, Murshidabad district, West Bengal, India. Foodstuffs tested included 8 kinds of vegetables, 4 kinds of cereals and bakery goods, and 4 kinds of spices collected from arsenic-affected areas. Concentration levels in terms of total mean for each heavy metal in the foodstuffs from the two districts were: 20.9 and 21.3 µg kg^{-1} (As) in vegetables; 130.0 and 179.00 µg kg^{-1} (As) in cereals and bakeries; 133 and 202 µg kg^{-1} (As) in spices, respectively. Generally, levels of Cu and Zn were higher in foodstuffs from Jalangi district than those from Domkal district. The opposite was found for foodstuffs from Domkal district regarding contamination levels of Mn. In all cases, spice commodities showed high Se contamination levels, accounting for 495 and 365 µg kg^{-1} for analyzed samples from Jalangi and Domkal districts, respectively.

13.4   Heavy metals in non-conventionally produced crops

In addition to crops produced by traditional farming systems followed for many thousands of years, other recent technologies have been developed and spread worldwide. Among these are genetically modified crops and organic food. We do not report on the benefits or risks of crops produced by such technologies, but we will attempt to focus on the quality of genetically modified and organically produced crops versus conventionally grown crops with respect to heavy metals content.

In their review article, Bagwan *et al.* (2010) concluded that genetically modified foods have the potential to solve many of the world's hunger and malnutrition problems, and to help protect and preserve the environment by increasing yield and reducing reliance upon chemical pesticides and herbicides. There remained many challenges ahead for governments however, especially in the areas of safety testing, regulation, international policy, and food labeling.

Many people feel that genetic engineering is the inevitable wave of the future and that we cannot afford to ignore a technology that has such enormous potential benefits. However, we must proceed with caution to avoid causing unintended harm to human health and the environment as a result of our enthusiasm for this powerful technology. Indeed, there are concerns about the safety of genetically modified crops. The concerns are that they may contain allergenic substances due to the introduction of new genes into crops. The genetically modified crops might contain other toxic substances (such as enhanced amounts of heavy metals) and the crops might not be substantially equivalent in genome, proteome, and metabolome compared to unmodified crops (Bakshi, 2003; Ekici and Sancak, 2011).

Sludge contains plant nutrients, but it cannot be used as a fertilizer because it is contaminated with toxic heavy metals. The purpose of creating some genetically modified crops is to utilize municipal sludge as fertilizer. However, the introduction of some genes into crop plants can remove heavy metals such as mercury or lead from the soil and concentrate them in the plants. The goal is to genetically engineer plants to localize those metals in inedible parts of plants to prevent adverse health effects from consumption of such crops. In tomato, for example, the metals would be sequestered in the roots; in potatoes, they will be sequestered in leaves. Turning on the genes in only some parts of the plants requires

the use of genetic 'on' and 'off' switches that turn on only in certain tissues, such as leaves. Such products pose risks of contaminating foods with high levels of toxic metals if the 'off' switch does not work properly in edible tissues (Cummins, 2000). It is important to keep in mind that crops used in heavy metal extraction should not be consumed as human food.

Within the last decade there have been a number of excellent reviews comparing organic with conventionally grown foods for quality claims (e.g. Arvanitoyannis and Krystallis, 2005; Bagwan *et al.* 2010; Ekici and Sancak, 2011). Many of the studies in these reviews have been carried out to compare the nutritional quality of organically grown foods with those produced by conventional methods. The conclusions of these reviews concerning food quality claims have been anything but corroborative.

Organically farmed vegetables such as cucumber, tomato, potato, and carrot have been sold in the Egyptian local markets for about a decade. Vegetables produced under greenhouse conditions were available in Egypt several decades ago. The Egyptian local market therefore contains a variety of vegetables produced by a range of methods, in addition to those produced from conventional or traditional cultivations under open field conditions.

As part of a large-scale monitoring program, pesticide residues and heavy metals were determined in cucumber fruits produced from conventional (C), greenhouse (G) and organic farming (O) to evaluate contamination levels and possible risks to human health. The monitoring program included also potato tubers from conventional (C) and organic (O) farming. For the purpose of this chapter, we focus on heavy metals contamination only. For full details of the studies, see Mansour *et al.* (2009a, b).

According to Mansour *et al.* (2009a), the majority of the analyzed samples of cucumber contained detectable concentrations of Zn, Cu, Mn, Fe, Cd, Pb, Cr, Ni, and Co. Only Pb and Cd were found in a number of samples at concentrations exceeding their maximum levels (MLs). Contamination among the three types of cucumber by heavy metals varied from one season to another.

Generally, the total heavy metal contamination in the three cucumber types (C, G, O) accounted to 4.968, 5.350 and 6.248 mg kg^{-1}, respectively. Figure 13.3 depicts the seasonal variation of heavy metal concentrations in different types of cucumber fruits collected from conventional,
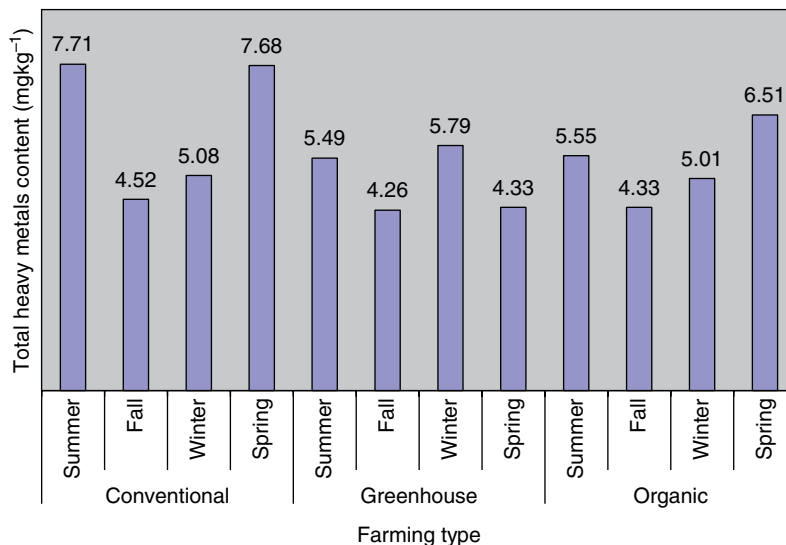

Figure 13.3 Seasonal variation of heavy metal concentrations in different types of cucumber fruits collected from conventional, greenhouse, and organic cultivations in Egypt. Data adapted from Mansour *et al.* (2009a).

greenhouse, and organic cultivations in Egypt. The pattern of contamination of cucumber by heavy metals in different seasons could be demonstrated in terms of general means of total metal contents in each season, and the percentage of each metal in these total metal contents as shown in Figure 13.4 and Table 13.1. The order of heavy metals contamination by seasons in C cucumber was: summer (7.71 mg kg^{-1}) > spring (7.68 mg kg^{-1}) > winter (5.08 mg kg^{-1}) > fall (4.52 mg kg^{-1}).

In a similar manner, the pattern of heavy metal contamination in the other two types of cucumber could be determined. Samples of G cucumber (Figure 13.3) belonging to the winter season contained the highest levels of heavy metals (5.794 mg kg^{-1}) while those of fall season showed the lowest value (4.257 mg kg^{-1}). In case of O cucumber (Figure 13.3), the metal contamination by different seasons showed the following order: spring (6.51 mg kg^{-1}) > summer (5.55 mg kg^{-1}) > winter (5.01 mg kg^{-1}) > fall (4.33 mg kg^{-1}).

Table 13.2 shows how much each metal percentage in the total metals was found in the analyzed samples. Iron (Fe) represented the highest constituent: it amounted to 48.43%, 41.98%, and 42.27% in C, G, and O cucumbers, respectively.

Figure 13.4   Seasonal variation of heavy metal content in conventionally and organically farmed potato tubers collected from Egyptian markets. Data adapted from Mansour *et al.* (2009b). For color details, see color plates section

Table 13.2   Total heavy metal content in different types of cucumber fruits collected from Egyptian markets, and percent of some metals in the total content. Data adapted from Mansour *et al.* (2009a).

Property	Conventional	Greenhouse	Organic
Total heavy metal content (mg kg^{-1})	6.25	4.97	5.35
Fe (%)	48.43	41.98	42.27
Zn (%)	22.35	28.67	26.29
Cu (%)	8.51	8.28	6.40
Pb (%)	4.04	5.76	5.81
Cd (%)	1.10	0.67	0.91

The highest contribution of Zn (28.67%) was estimated in G cucumbers, while the latter contained the lowest Cd percentage (0.67%). Copper recorded the lowest contribution (6.40%) in O cucumbers, while the Pb contribution (5.81%) was higher than in the two other cucumber types. Such variation of heavy metals as total, as well as their elemental constituents among the different types of cucumbers, may be a function of the practical and environmental conditions associated with the different farming systems used in production of the three types of cucumber. Heavy metal contamination may be a result of irrigation with contaminated water, the addition of fertilizers and metal-based pesticides, industrial emissions, transportation, harvesting process, storage, and/or sale. It is well known that plants take up metals by absorbing them from contaminated soils as well as from deposits on parts of the plants exposed to the air from polluted environments (Sharma *et al.*, 2008).

Regarding to heavy metals in potato tubers, Mansour *et al.* (2009b) reported that the majority of the analyzed samples contained detectable concentrations of Zn, Cu, Mn, Fe, Cd, Pb, Cr, Ni, and Co. Specifically, Pb and Fe were found in a number of samples at concentrations exceeding their MLs. Contamination among the two types of potatoes (C and O) varied from one season to another, and contamination of C potatoes was nearly twice that of O potatoes by heavy metals.

The pattern of contamination of potato samples by heavy metals in different seasons could be described in terms of general means of total metal contents in each season (Figure 13.4), and also the percentage of each metal in the total contents as shown in Tables 13.3 and 13.4. The order of heavy metals contamination by seasons in C potato was: fall (44.70 mg kg^{-1}) > spring (41.14 mg kg^{-1}) > winter (33.64 mg kg^{-1}) > summer (19.87 mg kg^{-1}). In case of O potato samples, the order of heavy metals contamination by seasons was: winter (32.96 mg kg^{-1}) > spring (18.46 mg kg^{-1}) > summer (10.99 mg kg^{-1}) > fall (7.76 mg kg^{-1}) (Figure 13.4).

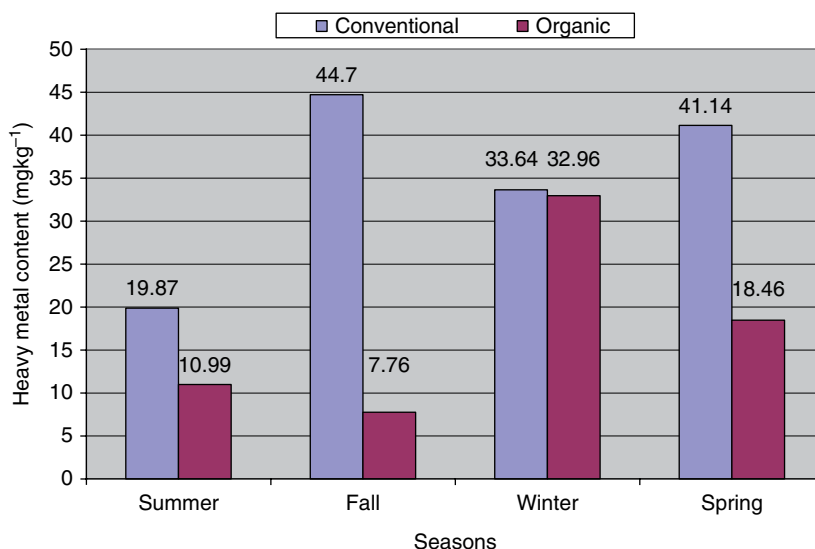**Table 13.3** Total heavy metal content in conventionally and organically farmed potato tubers collected from Egyptian markets, and percent of some metals in the total content. Data adapted from Mansour *et al.* (2009b).

Property	Conventional	Organic
Total heavy metal content (mg kg^{-1})	34.84	17.59
Fe (%)	83.60	68.48
Zn (%)	7.79	16.83
Cu (%)	2.70	4.50
Mn	1.95	4.14
Cr	2.45	3.01
Pb (%)	1.19	1.99
Cd (%)	0.10	0.30

Table 13.4 Estimated intake levels (µg per day) of lead and cadmium by vegetables and fruits based on global diet (182 and 236 g day^{-1} for vegetables and fruits, respectively). Source: Soliman *et al.*, 1997. Reproduced with permission of the authors. Estimated Pb and Cd intake level in vegetables: 9 and 2 µg day^{-1} and in fruits: 12 and 1 µg day^{-1}, respectively.

Vegetable/fruits	Pb (µg day^{-1})	Cd (µg day^{-1})	Vegetable/fruits	Pb (µg day^{-1})	Cd (µg day^{-1})
Spinach	54.60	2.184	Mallow	67.34	0.182
Chard	27.30	0.364	Radish	61.88	0.728
Garden rocket	32.76	7.644	Leek	56.42	3.094
Dill	32.76	0.364	Parsley	32.76	1.456
Coriander	27.30	0.346	Celery	47.32	0.546
Lettuce	20.02	0.546	Kale	29.12	1.092
Spearmint	32.66	0.182	Molokhia	118.30	1.638
Green onion	49.14	0.182	Potato tuber	25.48	0.910
Tomatoes	27.30	0.708	Strawberry	103.60	11.564
Peach	128.85	6.136	Cantaloupe	21.24	0.708

To compare the two types of potatoes with respect to total concentration of heavy metals, all the results obtained were computed to yield general means for summation of contaminants over 12 months. Contamination in C potatoes with total heavy metals accounted to 34.84 mg kg^{-1} in C potatoes, corresponding to 17.59 mg kg^{-1} in O potatoes (Table 13.3). This gives an indication that contamination of C potatoes was nearly twice the contamination of O potatoes by heavy metals. However, this was not the case for constituents of individual metals in the total contaminants. Only Fe constituted 83.60% of the total concentration of heavy metals found in C potatoes, compared to 68.48% in O potatoes. Other metals such as Zn, Cu, Mn, Pb, and Cd were found in higher percentages in O potatoes. For example, Zn accounted for 16.83% of total heavy metals found in O potatoes compared to 7.79% in the case of C potatoes (Table 13.3).

The estimation of dietary intake of heavy metals by consuming potatoes revealed that none of the studied heavy metals cause dietary intake risks to human health (Mansour *et al.*, 2009b). Several investigators in Egypt have determined heavy metals in potatoes (e.g. Soliman *et al.*, 1997; Dogheim *et al.*, 2004; Radwan and Salama, 2006); their results are comparable to the results of our study (Mansour *et al.*, 2009b).

A systematic survey of As, Cd, Cr, Cu, Ni, Pb, and Zn concentrations in vegetables from 416 samples (involving 100 varieties) in Beijing was carried out in order to assess the potential health risk to local inhabitants. The average concentrations were found to be 0.013, 0.010, 0.023, 0.51, 0.053, 0.046, and 2.55 μg g^{-1} fw, respectively. The Cu and Zn levels in all samples were within the Chinese National Standards, but the Pb, As, Ni, Cr, and Cd concentrations in 17.3%, 12.6%, 2.62%, 0.96%, and 0.58% of samples surpassed the standards. All elements except Zn in open-field vegetables and local-produced vegetables were all significantly higher than those in greenhouse vegetables and non-locally produced vegetables, respectively (Song *et al.*, 2009).

13.5   Dietary health risk assessment of heavy metals through consumption of food commodities

Heavy metals not only affect the nutritive values of vegetables but also affect the health of human beings; safe limits of these heavy metals are therefore lowered regularly in these vegetables. This regulation is the responsibility of national and international regulatory authorities (Mohammad and Ahmed, 2006). A health risk assessment of heavy metals by the population is useful for obtaining information about any threat regarding heavy metals contamination in vegetables. Different methods are used for health risk assessments by different researchers. Khan *et al.* (2009) reviewed different methods for assessment of the heavy metals concentration in the human body as a result of consuming contaminated vegetables and provided mathematical calculations for the metals Pb, Cu, Cd, and Zn. These methods include the daily intake of metals (DIM), daily dietary index (DDI), provisional tolerable daily intake (PTDI), along with the methods used for health risk assessment. The health risk assessment methods include hazard quotient (HQ) and health risk index (HRI).

The estimation of the risk associated with dietary intakes of heavy metals and pesticide residues by the consumer is a vital and integral part of regulatory processes. The exposure of the consumer is compared directly to the acceptable daily intake (ADI) for pesticides and to the tolerable daily intake (TDI) for heavy metals. The exposure is obtained using the basic equation:

$$\text{Exposure (mg kg}^{-1}\text{ bw day}^{-1})$$
$$= \text{Consumption (mg kg}^{-1}\text{ bw day}^{-1})$$
$$\times \text{Residue (mg kg}^{-1}).$$

The establishment of the ADI and the TDI is based on the results of toxicological studies that involve the determination of the lowest no-observed-adverse-effect level/10 (SF1) × 10 (SF2), where SF corresponds to safety factor. SF1 and SF2 account for interspecies and intraspecies variability, respectively. In order to evaluate the

risk to the consumer associated with the presence of heavy metals and pesticides in food, the level of contamination in European countries has been reviewed. The exposure of European consumers to lead, cadmium, arsenic, and mercury is greater than the TDI (Nasreddine and Parent-Massin, 2002). Approximately one-third of cadmium dietary intake is attributed to the ingestion of animal products, while plant products provide the remaining two-thirds. Shellfish and the kidneys of stock animals can accumulate significant amounts of cadmium from their surrounding environment (Ministry of Health, ESR 1995).

There are many methods for estimating the consumer-based health risk assessment, including provisional tolerable daily intake (PTDI). PTDI is a reference value established by WHO for cadmium (1992a) and lead (1992b). Mohammad and Ahmed (2006) stated that by this method the potatoes contributed 1 µg, 2 µg, 0.08 mg and 0.72 mg of Pb, Cd, Cu, and Zn, respectively, on the basis of the average 100 g/person/day consumption of potatoes. Similarly, for the vegetables diet/person/day is 116.7 g. They stated that if the mean concentrations of heavy metals (Pb, Cd, Cu, and Zn) remain the same (0.26, 0.04, 3.86, and 13.5), respectively in vegetables, they will contribute 30 µg, 4.67 µg, 0.45 mg, and 1.58 mg to the daily intake, respectively. They also concluded that PTDIs for the Egyptian population were lower than the safe limits by WHO/FAO (214 µg, 60 µg, 3 mg and 60 mg for Pb, Cd, Cu, and Zn, respectively).

13.5.1 Risk assessment

The risk of intake of metal-contaminated vegetables to human health is quantified by the hazard quotient (HQ). This is a ratio of determined dose to the reference dose (RfD). There will be no risk to the population if the ratio < 1; if the ratio is ≥ 1 then the population will experience a health risk. This risk assessment method has been used by several researchers (e.g. Chien $et\ al.$, 2002; Wang $et\ al.$, 2005) and demonstrated to be valid and true. HQ is defined:

$$HQ = W_{plant} \times M_{plant} / (RfD \times B)$$

where W_{plant} is the dry weight of contaminated plant material consumed (mg day^{-1}), M_{plant} is the plant concentration of metal in vegetables (mg kg^{-1}), RfD is the food reference dose for the metal (mg day^{-1}) and B is the body mass (kg). The values of RfD for heavy metals were taken from Integrated Risk Information System (Gholizadeh $et\ al.$, 2009) and Department of Environment, Food and Rural Affairs (DEFRA, 1999).

13.5.2 Daily dietary index

Since food crops are contaminated by heavy metals, their daily intake must be evaluated for comparison as given by US EPA. Daily dietary index (DDI) is defined:

$$DDI = X \times Y \times Z / B$$

where X is metal concentration in the vegetable (mg kg^{-1}); Y is dry weight of the vegetable (mg); Z is approximate daily intake (mg day^{-1}); and B is average body mass of the consumers (kg).

13.5.3 Daily intake of metals

Daily intake of metals (DIM) is determined via:

$$DIM = C_{metal} \times C_{factor} \times D_{intake} / B$$

where C_{metal} is the heavy metals concentration in plants (mg kg^{-1}); C_{factor} is the conversion factor to convert fresh vegetable weight to dry weight (Rattan $et\ al.$, 2005); and D_{intake} is daily intake of vegetables.

13.5.4 Health risk index

13.5.4.1 Estimation

Dietary risk assessment depends upon many sources of data and information on the level of a contaminant in specific food and the food consumption pattern of this food by different levels of consumers, which may differ greatly from one location to another in the same country. The problem is magnified when reliable comparative data are needed to set up an overview at global

level. For this reason, Luetzow (2003) explored the specific problems which would emerge if global intake assessments are requested; lack of representative regional data for consumption patterns and insufficient knowledge about levels of chemicals occurring in foods in many countries mean that exposure assessments do not provide risk managers with a true global picture. There is a need to improve the collection and dissemination of such data.

By using daily intake of metals (DIM) and reference oral dose we can derive the health risk index (HRI):

$$HRI = DIM / RfD.$$

If HRI < 1, then the exposed population is said to be safe (IRIS, 2003).

In their assessment of the risk to human health, Singh *et al.* (2010) determined metal pollution index (MPI) by calculating the geometrical mean of concentrations of the metals in the food items according to Usero *et al.* (1997):

$$MPI\left(\mu g\, g^{-1}\right) = Cf_1 \times Cf_2 \times \ldots \times Cf_n\right)^{1/n}$$

where Cf_n is the concentration of metal n in the sample. The health risk index (HRI) was then calculated as the ratio of estimated exposure of test crops and oral reference dose (Cui *et al.*, 2004). Oral reference doses were 4×10^{-2}, 0.3, and 1×10^{-3} $mg\,kg^{-1}\,day^{-1}$ for Cu, Zn, and Cd, respectively (USEPA, 2002) and 0.004, 0.02, and 1.5 $mg\,kg^{-1}$ day^{-1} for Pb, Ni, and Cr, respectively (USEPA, 1997). Estimated exposure is obtained by dividing daily intake of heavy metals by their safe limits. An index of >1 is considered as unsafe for human health (USEPA, 2002).

Daily intake of metal (DIM) was calculated via:

$$DIM = C_{metal} \times D_{intake} / B$$

where C_{metal}, D_{intake} and B represent the heavy metal concentrations in plants ($\mu g\, g^{-1}$), daily intake of vegetables and average body weight, respectively. The average daily vegetable intake rate was calculated by conducting a survey of 100 people with average body weight of 60 kg who were asked for their daily intake of particular vegetables from the experimental area in each month of sampling (Ge, 1992; Wang *et al.*, 2005).

Harmanescu *et al.* (2011) compared the levels of heavy metals (Fe, Mn, Zn, Cu, Ni, Cd, and Pb) found in common vegetables (parsley, carrot, onion, lettuce, cucumber, and green beans) grown in contaminated mining areas in Romania with those grown in a reference clear area and estimated their potential detrimental effects via calculation of the daily metal intake (DIM) and target hazard quotients (THQ) for normal daily consumption of these vegetables, for both male and female genders. The results of this study regarding metal contents in soils, vegetables, DIM and THQ suggested that the consumption of some vegetables (especially parsley, carrot, and cabbage and less for lettuce, cucumber, and green beans) is not free of risks in these areas.

Yang *et al.* (2011) estimated daily intake (DI) of heavy metals (Pb, Zn, Mn, Cu, Cd, and Cr) in market vegetables in Chongqing, China and their potential health risk for local consumers by calculating the target hazard quotient (THQ). The results showed that the measured Pb and Cd concentrations exceeded the safety limits given by FAO/WHO and Chinese regulations, indicating serious contamination of market vegetables by these metals. As DI values for Pb, Mn, and Cd were also above the international guideline bases, the health risk to the consumer is obvious. The individual THQ for Pb and Cd in pakchoi and Cd in mustard, and the combined THQ for all metals in each vegetable species excluding lettuce, were above the threshold 1.0, implying an adverse effect on health. The authors stated that attention should be paid particularly to the potential hazardous exposure to vegetable heavy metals, especially for Pb and Cd, over a lifetime for people in Chongqing.

13.5.4.2   Relevant studies

Vegetables and cereal crops from a wastewater-irrigated site in India were analyzed for heavy metals (Cd, Cu, Pb, Zn, Ni, and Cr) to assess the risk to human health due to consumption of these foods (Singh *et al.* 2010). Heavy metal concentrations

were several-fold higher in all the collected samples compared to clean-water-irrigated samples. Cd, Pb, and Ni concentrations were above the 'safe' limits of Indian and WHO/FAO standards in all the vegetables and cereals. The higher values of metal pollution index and health risk index indicated heavy metal contamination in the wastewater-irrigated site, presenting a significant threat of negative impact on human health. Rice and wheat grains contained less heavy metals as compared to the vegetables, but the health risk was greater due to the higher contribution of cereals to diet. The study suggests that wastewater irrigation led to accumulation of heavy metals in foodstuff, causing potential health risks to consumers.

Based on Singh *et al.* (2010) results, Figure 13.5 illustrates health risk index (HRI) of cadmium and lead in selected foodstuffs. HRI≥1 is indicative of a health risk to human. Orisakwe *et al.* (2012) assessed lead, cadmium, and nickel levels in food crops, fruits, and soil samples from Ohaji, Umuagwo, and Owerri in SE Nigeria and estimated the potential health risks of metals to the general population. Concentrations of Pb, Cd, and Ni in Ohaji exceeded the maximum allowable limits for agricultural soil as recommended by the EU. Lead, Cd, and Ni in the food crops were highest in *Oryza sativa*, *Glycine max*, and *Pentabacta microfila*, respectively. Highest levels of Pb, Cd, and Ni in fruits were detected in *Canarium schweinfurthii*, *Citrus reticulate*, and *Ananas comosus*, respectively. The true lead and cadmium intake for the rice-based meal were 3.53 and 0.034 g kg^{-1}, respectively, whereas the true intake of lead and cadmium for the cassava-based meal were 19.42 and 0.049 g kg^{-1}, respectively. Generally, it can be deduced that metal contamination in the studied matrices seemed to be higher in food crops than farm soils and fruits in the case of Pb and Ni. Cd was higher in farm soils than in the plant commodities.

In Nigeria, Tsafe *et al.* (2012) evaluated the heavy metal uptake of vegetables grown in Yargalma in northern Nigeria and assessed risks
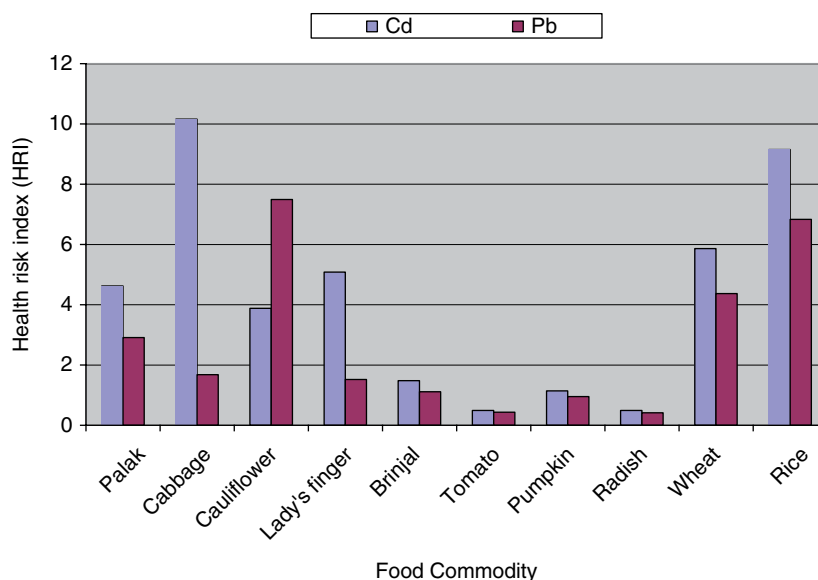
Figure 13.5   Health risk assessment for cadmium and lead via intake of foodstuffs from wastewater-irrigated sites in India; based on estimated health risk index (HRI). HRI≥1 indicates a health risk to humans. Analyzed vegetables and cereal crops: palak: *Beta vulgaris* L.; cabbage: *Brasssica oleracea* L.; cauliflower: *Brassica oleracea* L.; lady's finger: *Abelmoschus esculentus* L.; Brinjal: *Solanum melongena* L.; tomato: *Lycopersicon esculentum* L.; pumpkin: *Cucurbita maxima* Duch.; radish: *Raphanus sativus* L.; wheat: *Triticum aestivum* L.; rice: *Oryza sativa* L. Adapted from Singh *et al.* (2010). For color details, see color plates section

involved in the consumption of such vegetables. The results revealed the trend in soil metals concentration is Al>Fe>Mn>Mg>Zn>Pb>Ni>Cr>Co>Cu>Cd, and for the plant the trend is Fe>Mn>Mg>Zn>Al>Co>Ni>Pb>Cr>Cu>Cd. The transfer pattern for metals from soil to plant is Co>Cu>Cd>Mg>Ni>Zn>Mn>Fe>Pb>Cr>Al. The trend of the daily intake of metal (DIM) value was Fe>Mn>Mg>Zn>Al>Co>Ni>Pb>Cr>Cu>Cd. The trend in daily dietary intake (DDI) was found to be slightly different: Al>Fe>Mn>Mg>Zn>Pb>Ni>Cr>Co>Cu>Cd. The results showed very high health risk index (HRI) values for Cd (65.38), Zn (11.48) and Cu (2.09). This confirmed that the soil and vegetables in the area were contaminated with the assayed metals.

Dietary intake is the main route of exposure for most people, although inhalation can play an important role in very contaminated sites (Tripathi *et al.*, 1997). Zhuang *et al.* (2009) investigated heavy metal (Cu, Zn, Pb, and Cd) concentrations in soils and food crops and estimated the potential health risks of metals to humans via consumption of polluted food crops grown at four villages around the Dabaoshan mine, south China. The heavy metal concentrations in paddy and garden soils exceeded the maximum allowable concentrations for Chinese agricultural soil. The paddy soil at Fandong village was heavily contaminated with Cu (703 mg kg^{-1}), Zn (1100 mg kg^{-1}), Pb (386 mg kg^{-1}) and Cd (5.5 mg kg^{-1}). Rice tended to accumulate higher Cd and Pb concentration in grain parts. The concentrations of Cd, Pb, and Zn in vegetables exceeded the maximum permissible concentration in China. Taro grown at the four sampled villages accumulated high concentrations of Zn, Pb, and Cd.

Bioaccumulation factors for heavy metals in different vegetables showed a trend in the order: Cd>Zn>Cu>Pb. Bio-accumulation factors of heavy metals were significantly higher for leafy than for non-leafy vegetables. The target hazard quotient (THQ) of rice at four sites varied within the range 0.66–0.89 for Cu, 0.48–0.60 for Zn, 1.43–1.99 for Pb, and 2.61–6.25 for Cd. Estimated daily intake (EDI) and THQs for Cd and Pb of

rice and vegetables exceeded the FAO/WHO permissible limit. The authors concluded that heavy metal contamination of food crops grown around the mine posed a great health risk to the local population through consumption of rice and vegetables.

Based on the results of heavy metal concentrations in different vegetables from villages in the vicinity of Dabaoshan mine, south China (Zhuang *et al.*, 2009), it is possible to compare the total heavy metal contamination in 15 plant species (vegetables) from 3 villages (Zhongxin (ZX), Fandong (FD) and Shangba (SB)). The results reveal total Cu content of 18.24, 16.01, and 19.38 mg kg^{-1} in vegetables from ZX, FD, and SB villages, respectively. Zn amounted to 129.98, 183.88, and 141.83 mg kg^{-1}; Pb 2.57, 2.40, and 2.73 mg kg^{-1}; and Cd 3.17, 2.93, and 3.01 mg kg^{-1}.

Cadmium (Cd), mercury (Hg), arsenic (As), lead (Pb), and tin (Sn) concentrations were determined in soft tissues (wet weight) from whole greenshell mussels (*Perna canaliculus*) collected from Urapukapuka–Rawhiti Island, Opua Marina, Waitangi Bridge, and Opua Wharf from the Bay of Islands, northern New Zealand (NZ) (Whyte *et al.*, 2009). All samples had metal concentrations well below the Food Standards Australia and New Zealand (FSANZ) maximum limits and were comparable to, or less than, concentrations observed in previous NZ studies. Accumulation of the measured metals differed from one location to another; mussels from Urapukapuka location contained the highest Cd and As values (0.75 and 2.97 mg kg^{-1}, respectively). Based on the average values detected in the current study, the concentrations of heavy metals ingested in a 'typical' diet containing greenshell mussels are below the provisional tolerable weekly intake (PTWI). However, Māori (indigenous people of New Zealand), Pacific Islanders and Asians consume a far greater quantity of seafood (and therefore heavy metals) than the general public of New Zealand and could potentially consume enough shellfish to exceed the PTWI for Cd (but not for Hg, As, Pb, or Sn).

Although present results based on the current PTWIs indicate no significant health risk to

greenshell mussel consumers in this region, PTWIs change over time; concentrations which were thought to be safe are later found to be harmful. Additionally, differences in individual human susceptibilities to various toxins could increase the risk of harm for consumers with low tolerance to heavy metals.

Food grown on sewage-irrigated soils along the Musi River and its environs in India were assessed for heavy metals (Zn, Cr, Cu, Ni, Co, and Pb) contamination in soils, forage grass, milk from cattle, and leafy and non-leafy vegetables. Partitioning pattern of soil revealed high levels of Zn, Cr, and Cu associated with labile fractions, making them more mobile and plant available. The associated risk was assessed using hazard quotient (HQ). Human risk was assessed in people known to consume these contaminated foods by analyzing metals concentrations in venous blood and urine. Results showed high amounts of Pb, Zn, Cr, and Ni relative to permissible limits. HQ was found to be high for Zn, followed by Cr and Pb with special reference to leafy vegetables, particularly spinach and amaranthus (Chary *et al.*, 2008).

Khan *et al.* (2008) studied the health risks of heavy metals in contaminated food crops irrigated with wastewater. Results indicated that there is a substantial build-up of heavy metals in wastewater-irrigated soils collected from Beijing, China. Heavy metal concentrations in plants grown in wastewater-irrigated soils were significantly higher ($P < 0.001$) than in plants grown in the reference soil, and exceeded the permissible limits set by the State Environmental Protection Administration (SEPA) in China and the World Health Organization (WHO). Furthermore, this study highlighted that both adults and children consuming food crops grown in wastewater-irrigated soils ingest significant amounts of the metals studied. An HRI<1 however indicates a relative absence of health risks associated with the ingestion of contaminated vegetables.

Concentrations of heavy metals (Hg, As, Cr, Cu, Ni, Pb, Zn, and Cd) in wheat grains were investigated in different areas of a developed industry city in SE China (Kunshan city), and their potential risk to health of inhabitants was estimated (Huang *et al.*, 2008). The Zn, Cr, Ni Cd, and Hg concentrations of several soil samples exceeded the permissible limits of the Chinese standard. In addition, concentrations of heavy metals in wheat grain decreased in the order of Zn > Cu > Pb > Cr > Ni > Cd > As > Hg. There were some wheat samples whose Zn, Pb, and Cd concentrations exceeded the permissible limits. In relation to non-carcinogenic risks, the hazard quotient (HQ) of individual metals indicated values within the safe interval.

The health risk due to the added effects of eight heavy metals was significant for rural children and rural adults, but not for urban adults and urban children. HQ (individual risk) and HI (hazard index of aggregate risk) to different inhabitants due to heavy metals followed the same sequence of: country children > country adults > urban children > urban adults.

Among the heavy metals, potential health hazards due to As, Cu, Cd, and Pb were great and that due to Cr was a minimum. It was suggested that more attention should be paid to the potential added threat of heavy metals to the health of country inhabitants (both children and adults) through consumption of wheat in Kunshan (Huang *et al.*, 2008).

Maleki and Zarasvand (2008) determined the levels of four different heavy metals (Cd, Pb, Cr, and Cu) in various vegetables – for example, leek (*Allium ampeloprasum*), sweet basil (*Ocimum basilicum*), parsley (*Petroselinum crispum*), garden cress *(Lepidium sativum)* and tarragon (*Artemisia dracunculus*) – cultivated around Sanandaj city, Iran. The contributions of the vegetables to the daily intake of heavy metals from vegetables were investigated. The average concentrations of each heavy metal regardless of the kind of vegetable for Pb, Cu, Cr, and Cd were 13.60 ± 2.27, 11.50 ± 2.16, 7.90 ± 1.05, and $0.31 \pm 0.17 \, \text{mg kg}^{-1}$, respectively. Based on the above concentrations and data from the National Nutrition and Food Research Institute of Iran, the dietary intake of Pb, Cu, Cr, and Cd through vegetable consumption was estimated to be 2.96, 2.50, 1.72 and $0.07 \, \text{mg day}^{-1}$, respectively. This led to the conclusion that vegetables grown

in that region are a health hazard for human consumption.

The target hazard quotients (THQs) and hazard index (HI) were calculated to evaluate the non-carcinogenic health risk from individual heavy metal and combined heavy metals due to dietary intake (Zheng *et al.*, 2007). Target hazard quotients for individual heavy metals by consuming foodstuffs in the industrial area of Huludao, China were all <1, indicating that the health risk associated with the intake of a single heavy metal through consumption of only one kind of foodstuff (e.g. vegetable) was absent. However, consumption of all foodstuffs would lead to potential health risks for children and adults, since hazard indexes (HIs) for heavy metals due to dietary intake were >1. The relative contributions of Hg, Pb, Cd, Zn, and Cu to the HIs were 1.7%, 11.7%, 24.0%, 23.4%, and 39.6% for adults, and 1.5%, 11.7%, 21.8%, 26.1%, and 38.8% for children. Cereal, seafoods, and vegetables were the main sources of heavy metal intake from foodstuffs for adults and children, but fruit, milk, beans, and eggs were secondary contributors.

Li *et al.* (2006) conducted a field survey to investigate the metal and arsenic contamination in soils and vegetables in four villages located in Baiyin, China and to evaluate the possible health risks to local population through the food chain. Generally, the leafy vegetables were more heavily contaminated than non-leafy vegetables. Furthermore, the estimated daily intake amounts of the considered toxic elements (Cd, Pb, and Cu) from the vegetables exceeded the recommended dietary allowance levels. Vegetables grown in three villages were therefore seen to be affected by mining and smelting and were considered a health hazard for human consumption.

The daily intake of 12 metals (Na, K, Ca, Cu, Zn, Fe, Mn, Mg, Pb, Cd, Co, and Ni) by Mumbai adult population were assessed by analyzing duplicate diet samples (Raghunath *et al.*, 2006). A total of 250 diet samples containing 170 vegetarian diets and 80 non-vegetarian diets collected during April 2003 to March 2004 were analyzed during this study. Daily dietary intakes of 2.4 g Na and 1.2 g K were observed for Mumbai adults.

Daily dietary intakes of Ca, Cu, Zn, Fe, Mn, and Mg were 367, 1.0, 6.3, 6.7, 2.0, and 304 mg, respectively. Pb, Cd, Co, and Ni daily intakes by Mumbai adults were 32.3, 2.2, 2.2, and 108 mg, respectively. From this study it was observed that the intake of toxic metals such as Pb, Cd, and Ni is much lower than the tolerable daily intake derived from PTWI given by FAO/WHO, and could not be considered harmful in this group of subjects. Daily intake of the studied 12 metals was found to be higher in non-vegetarian diet compared to the vegetarian diet.

From heavy metal contaminants analysis of 20 different species of vegetables and fruits collected from a local market in Cairo, Egypt, Soliman *et al.* (1997) estimated the mean daily intake of lead in the analyzed vegetables and fruits to be 20.02–118.30 and 21.24–128.85 μg day^{-1}, respectively. Those for cadmium were 0.182–7.64 and 0.708–11.56 μg day^{-1} (Table 13.4). The authors concluded that the lead values were higher than those recorded by global diet limits for vegetables and fruits, which were 9.0–12.0 μg day^{-1}. In contrast, the levels of cadmium were lower than the global diet limits for vegetables and fruits which range from 1.0 to 2.0 μg day^{-1} in the different samples analyzed, with the exception of spinach, garden rocket, leek, strawberry, and peach where their estimated intake levels (μg day^{-1}) reached 2.184, 7.644, 3.094, 11.564, and 6.136, respectively (Table 13.4).

13.6   Conclusion

Contamination of foods by heavy metals has become an inevitable challenge these days. Air, soil, and water pollution are contributing to the presence of various harmful elements in foodstuff. The occurrences of heavy-metals-enriched ecosystem components arise from rapid industrial growth, advances in agricultural chemicalization, or the urban activities of human beings (Zukowska and Biziuk, 2008). The consumption of heavy-metal-contaminated food can seriously deplete essential nutrients in the body, causing a decrease in immunological defenses, intrauterine

growth retardation, impaired psycho-social behaviors, disabilities associated with malnutrition, and a high prevalence of upper gastrointestinal cancer (Arora *et al.,* 2008). Dietary risk assessment depends upon many sources of data and information on the level of a contaminant in specific food and the food consumption pattern of this food by different levels of consumers, which may differ greatly from one location to another in the same country. Therefore, risk/safety evaluation of dietary exposure to heavy metals should be regulated through governmental authorities at national and international levels.

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14

Heavy Metal Contamination as a Global Problem and the Need for Prevention/Reduction Measurements

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Summary

The published literature offer extensive data on heavy metals contamination in a variety of environmental components and foodstuffs. These elements are naturally present in our environment; human activities as well as the specific properties of heavy metals play an important role in their emission, transportation, accumulation, and free travel through the food chain. Prevention and/or reduction strategies for securing food safety and human health should therefore be integrated at local, regional, and global levels.

14.1   Introduction

Increasing industrialization has been accompanied throughout the world by the extraction and distribution of mineral substances from their natural deposits. Many of these have undergone chemical changes through technical processes and finally pass, finely dispersed and in solutions, by way of effluent, sewage, dumps, and dust, into the water, the earth, and the air and hence into the food chain. These include heavy metals relevant to this document. Together with essential nutrients, plants and animals also take up small amounts of contaminant heavy metal compounds and can concentrate them. As certain

heavy metals such as lead, cadmium, mercury, and arsenic (arsenic is usually regarded as a hazardous heavy metal even though it is actually a semi-metal) have been recognized to be potentially toxic within specific limiting values, a considerable potential hazard exists for human (Anonymous, 2003).

Heavy metals are natural components of the Earth's crust and cannot be degraded nor destroyed. They enter the human body through food, water, and air and are widely distributed in all environmental components. Focusing on vegetables and fruits, contamination can result from soil, irrigation water loaded by heavy metals, application of fertilizers and pesticides containing heavy metals, deposition of heavy metal particulates from air on plantations, industrials emissions, transportation, the harvest process, storage, and/or at the point of sales (Maleki and Zarasvand, 2008). Heavy metal pollution of surface and underground water sources also results in considerable soil pollution, and pollution increases when mined ores are dumped on the ground surface for manual dressing (Garbarino *et al.* 1995).

When agricultural soils are polluted, these metals are taken up by plants and consequently accumulate in their tissues. Animals that graze on such contaminated plants and drink from polluted waters, as well as marine lives that breed in heavy-metal-polluted waters, also accumulate such metals in their tissues and milk, if lactating (Garbarino *et al.* 1995; Osakwe, 2010). Humans are in turn exposed to heavy metals by consuming contaminated plants and animals, and this has been known to result in various biochemical disorders.

In summary, all living organisms within a given ecosystem are variously contaminated along the cycles of the food chain. Plants absorb a number of elements from soil, some of which have no known biological function and some are known to be toxic at low concentrations. As plants constitute the foundation of the food chain, some concerns have been raised about the possibility of toxic concentrations of certain elements being transported from plants to higher strata of the food chain. How heavy metals find their ways to the human body will be the focus of this document.

14.2 Pathway of heavy metals through the food chain

Special attention has been given to the uptake and biotransformation mechanisms occurring in plants, their role in bioaccumulation, and impact on consumers, especially human beings. Peralta-Videa *et al.* (2009) reviewed plant uptake of the toxic elements arsenic, cadmium, chromium, mercury, and lead and their possible transfer to the food chain. These elements were selected because they are well established as being toxic for living systems and their effects in humans have been widely documented.

Zhuang *et al.* (2009) investigated the accumulation and transfer of Pb, Zn, Cu, and Cd along a soil–plant–insect–chicken food chain at contaminated sites in China. The study site near a Pb/Zn mine was severely contaminated by heavy metals. Cd and Pb concentrations steadily declined with increasing trophic level ($p < 0.01$), but concentrations of Zn and Cu slightly increased from plant to insect larva ($p > 0.05$). The concentrations of heavy metals were the highest in chicken muscle, with lower values in liver and blood. The bioaccumulation of Pb was observed in chicken livers. The eliminations of Pb, Zn, Cu, and Cd via insect and chicken feces avoid metal bioaccumulation in insect and chicken body. These results suggest that the accumulation of heavy metals in specific animal organs or tissues could not be neglected, although transfer of metals to chicken from plant and insect was limited.

The accumulation of heavy metals (Cd, Pb, Hg, and Cr) was measured in water, sediment, plankton, and fish samples collected from Lake Beyşehir (Turkey) and irrigation and drinking water sources (Altindağ and Yiğit, 2005). In Lake Beyşehir, the accumulation orders of heavy metals were Cd > Pb > Cr > Hg in water, Pb > Cd > Cr > Hg in sediment, Pb > Cd > Cr > Hg in plankton, and Cd > Pb > Cr > Hg in the muscles and gills of chub, carp, tench; for the muscle of pikeperch, the accumulation order was Pb > Cd > Cr > Hg ($p < 0.05$). In addition, accumulation orders of heavy metals in the food web was also found to be water > plankton > sediment > fish tissues, except for Cr. According

to international criteria and Turkish regulations, heavy metal concentrations, especially Cd and Pb, in Lake Beyşehir were markedly above the permissible levels for drinking water (Altindağ and Yiğit, 2005).

Cui *et al.* (2011) determined nine heavy metals in samples of water, sediments, and aquatic organisms in the newly formed wetlands of the Yellow River Delta (YRD) of China to evaluate their concentrations and trophic transfer in food webs. The stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes were used to investigate trophic interactions. Results showed that most of the heavy metals detected in water and sediments were lower than that in the Yangtze River Delta and Pearl River Delta.

The longest food web is approximately four with the highest trophic level of birds. The difference in heavy metal concentrations between endangered Saunders's Gull and another three kinds of protected birds was not obvious. Cd, Zn, and Hg were identified to have an increase with the trophic level (TL), while As, Cr, Cu, Mn, Ni, and Pb showed an opposite trend; however, the biomagnification of the selected nine heavy metals in the food webs was not significant.

In this respect, data results and reports demonstrating the contribution of different sources of heavy metal contamination are provided in Sections 14.2.1–14.2.4.

14.2.1   Transfer of heavy metals from soil to vegetables

Most of the heavy metals are the natural constituents of the Earth's crust, from which they are taken by plants and transferred to the food chain. These metal concentrations vary from soil to soil. The concentration of metals in vegetables mainly depends on the texture of the soil or media on which they grow, but this also depends on the type and nature of plant (Kabata-Pendias and Pendias, 1984). Cultivation of crops for human or livestock consumption on contaminated soil can potentially lead to the uptake and accumulation of trace metals in the edible plant parts with a resulting risk to human and animal health (Gupta and Gupta, 1998; Monika and Katarzyna, 2004; McBride, 2007).

Islam *et al.* (2007) reviewed the phytotoxic effects and bioaccumulation of heavy metals in vegetables and food crops and assessed soil heavy metal thresholds for potential dietary toxicity. They reported that soil threshold for heavy metal toxicity is an important factor affecting soil environmental capacity of heavy metal and determines heavy metal cumulative loading limits. For the soil–plant system, heavy metal toxicity threshold is the highest permissible content in the soil (total or bio-available concentration) that does not pose any phytotoxic effects or heavy metals in the edible parts of the crops which exceed food hygiene standards. Factors affecting the thresholds of dietary toxicity of heavy metal in the soil–crop system include: soil type, which includes soil pH, organic matter content, clay mineral, and other soil chemical and biochemical properties; and crop species or cultivars regulated by genetic basis for heavy metal transport and accumulation in plants. In addition, the interactions of soil–plant-root–microbes play important roles in regulating heavy metal movement from soil to the edible parts of crops. Agronomic practices such as fertilizer and water management as well as crop rotation systems can affect bio-availability and crop accumulation of heavy metals, thus influencing the thresholds for assessing dietary toxicity of heavy metals in the food chain.

Wastewater irrigation, solid waste disposal, sludge applications, vehicular exhaust, and industrial activities are the major sources of soil contamination with heavy metals. Vegetables cultivated in contaminated soils take up heavy metals in large quantities, enough to cause potential health risks to the consumers (Cui *et al.*, 2005; Liu *et al.*, 2005; Yang *et al.*, 2007; Cârlig and Macoveanu, 2008; Khan *et al.*, 2008; Lăcătuşu *et al.*, 2009; Damian *et al.*, 2010; Singh *et al.*, 2010). In order to assess the health risks, it is necessary to identify the potential of a source to introduce risk agents into the environment, to estimate the amount of risk agents that come into contact with the human–environment

boundaries, and to quantify the health consequence of the exposure (Khan *et al.*, 2008).

Plants take up essential and non-essential elements from soils in response to concentration gradients induced by selective uptake of ions by roots, or by diffusion of elements in the soil. The level of accumulation of elements differs between and within species (Huang and Cunningham, 1996). Baker (1981) suggested that plants could be classified into three categories: (1) *excluders*: those that grow in metal-contaminated soil and maintain the shoot concentration at low level up to a critical soil value above which relatively unrestricted root-to-shoot transport results; (2) *accumulators*: those that concentrate metals in the aerial part; and (3) *indicators*: where uptake and transport of metals to the shoot are regulated so that internal concentration reflects external levels, at least until toxicity occurs.

As vegetables are the source of human consumption, so the soil-to-plant transfer quotient is the main source of human exposure. A convenient way for quantifying the relative differences of bioavailability of metals to plants is the transfer quotient (Cui *et al.*, 2004). The transfer quotient for Cd and Cu were higher than for Pb and Fe. The higher transfer quotient of heavy metal indicates the stronger accumulation of the respective metal by that vegetable (Khan *et al.*, 2008). A transfer quotient of 0.1 indicates that the plant is excluding the element from its tissues (Thornton and Farago, 1997). The greater the transfer coefficient value above 0.50, the greater the chances of vegetables for metal contamination by anthropogenic activities, indicating that environmental monitoring of the area is required (Sponza and Karaoglu, 2002).

Leafy vegetables accumulate much higher contents of heavy metals as compared to other vegetables. This is because leafy vegetables have a higher translocation and transpiration rate as compared to other vegetables, in which transfer of metals from root to stem and then to fruit is longer which results in lower accumulation than leafy vegetables (Itanna, 2002). The trend of transfer of these metals was of the order Cd > Cu > Pb > Fe (Khan *et al.*, 2008).

In Lebanon, Al-Chaarani *et al.* (2009) monitored the levels of four selected toxic heavy metals (lead, cadmium, chromium, and arsenic) in a total of 181 vegetable samples of which 66 are leafy vegetables, 84 are over ground vegetables, and 31 are underground grown vegetables. Overall, the levels ranged from non-detectable to 3.0904 μg g^{-1} for Pb, 0.0137–3.6170 μg g^{-1} for Cd, non-detectable to 19.55 μg g^{-1} for Cr, and non-detectable to 0.0636 μg g^{-1} for As, where μg g^{-1} refers to the weight of the metal per gram of dry sample weight. In all samples, the leafy vegetables contained considerably higher levels for all metals as compared to overground or underground vegetables. Figure 14.1 shows that the highest concentrations of Cr and Pb were found in mint, while spinach retained the highest Cd concentration value. Contamination levels with As were generally very low and not included in Figure 14.1.

A total of 104 canned soft drinks collected from several regions in Turkey were analyzed. The purpose of this study was to determine the levels of heavy metals in the drinks commonly consumed in Turkey. The results revealed that arsenic, copper, zinc, cadmium, and lead mean levels found in all soft drinks were within the Turkish Food Codex (TFC) values (Bingöl *et al.*, 2010).

14.2.2   Heavy metal transfer through irrigation water

In aquatic systems, the natural concentrations of metal ions are principally dependent on the ambient distribution, weathering, and leaching of these elements from the soil in the catchment area. Human activities, such as industrial and traffic emissions and various land-use practices, may increase heavy metal loading into aquatic ecosystems (Nriagu and Pacyna, 1988; Tarvainen and Kallio, 2002). Heavy metals are carried to the lakes and rivers through atmospheric deposition and/or discharge. The characteristics of the water, such as acidity or the amount of organic matter, are known to be important factors in determining the fate of heavy metals in lakes and rivers (Mannio *et al.*, 1995; Skjelkvåle *et al.*, 2001).
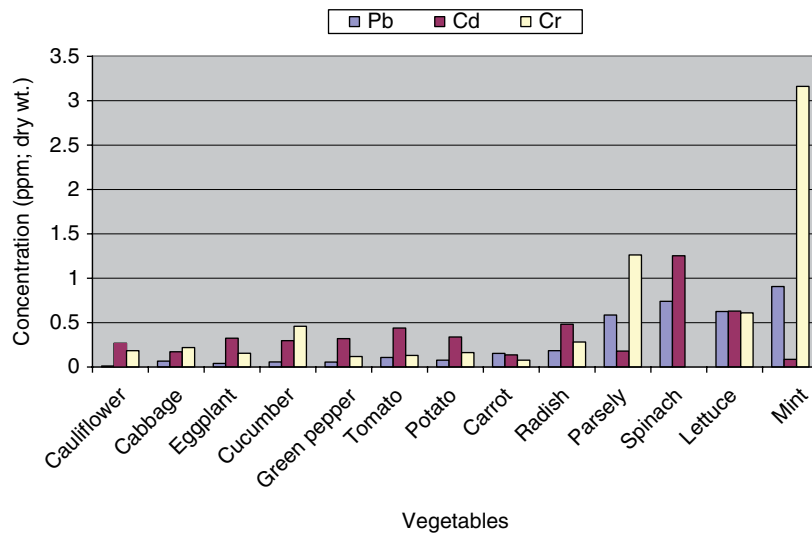
Figure 14.1 Concentration levels (ppm dry weight) of selected heavy metals in different vegetable samples grown and sold in Lebanon. Adapted from Al-Chaarani *et al.* (2009). For color details, see color plates section.

Aweng *et al.* (2011) evaluated the levels of selected heavy metals in irrigation water, soils, and selected fruit vegetables which were cultivated in coastal village and industrial area (light and medium industry) in Kota Bharu, Kelantan, Malaysia. Results obtained showed that water used for irrigation at both sites had the highest concentration of boron followed by manganese, iron, and other parameters. High concentrations of heavy metals in soil and irrigation water led to the accumulation of heavy metals in vegetables. Heavy metal concentrations varied among the test vegetables, which reflect the differences in their uptake capabilities and their further translocation to edible portion of the plants. The trend revealed that high concentrations of Cd, Pb, and Zn in soil and water led to high concentrations in vegetables, even though the concentrations in all vegetables were below the national and international permissible limits. Irrigation water was a dominant factor to determine the concentrations of heavy metal in vegetables compared to soil because irrigation water normally led to the accumulation of heavy metals in soil and consequently into vegetables.

Based on the results, it showed that boron was found to be the highest in irrigation water as well as in vegetables, but not in soil (Aweng *et al.*, 2011).

14.2.3 Heavy metals transfer and accumulation in fish

Heavy metals entering the water body are absorbed in sediments, and subsequently might migrate as a result of exchanges between water, sediment, and biota through biological and chemical process. In the 1960s, serious mercury pollution occurred in Sweden (Jernelöv *et al.*, 1975), and was later found in Canada (Wheatley, 1997). Following these events, researchers began to pay more attention to pollution by heavy metals. Accumulation of heavy metals in fish results primarily from surface contact with the water, by breathing, and via the food chain. Uptake by these three routes depends on the environmental levels of heavy metals in the habitat of the fish.

In recent years, the rapid development of industry and agriculture has resulted in increased pollution of heavy metals which are a significant environmental hazard for invertebrates, fish, and

humans. A significant amount of heavy metals are discharged into rivers resulting in sublethal effects or death in local fish populations (Megeer *et al.*, 2000; Xu *et al.*, 2004). The sediments in rivers and lakes play a significant role in determining water quality. Suspended sediments adsorb pollutants from the water, thus lowering the concentration of pollutants in the water column. Benthic sediments also provide habitat and a food source for benthic fauna. Pollutants may be directly or indirectly toxic to the aquatic flora and fauna. Given the detrimental effects of pollutants, many researchers have studied their effects on aquatic flora and fauna (Morrissey and Edds, 1994; Chen *et al.*, 2002; de Mora *et al.*, 2004).

The consumption of fish is recommended because it is a good source of omega-3 fatty acids, associated with health benefits due to its cardio-protective effects. However, the content of heavy metals discovered in some fish makes it difficult to clearly establish the effect of fish consumption on a healthy diet. Castro-González and Méndez-Armenta (2008) reviewed concentration levels of Hg, Cd, Pb, and As in several kinds of fish from different countries such as Spain, Croatia, USA, Egypt, and Slovakia. Such data could be used to assess contamination levels with respect to the maximum permissible limits set by FAO/WHO Expert Committee on Food Additives.

More than 700 composed fish organ samples (e.g. bone, brain, flesh, gills, gonad, liver) were collected from six governorates (Damietta, Port-Said, Hourghada, Suez, Ismaelia, Alexandria) to assess distribution of heavy metals in Egyptian fish

(Gomaa *et al.*, 1995). The brain was found to contain the highest concentrations of the analyzed metals (lead, cadmium, chromium, zinc, copper, manganese, iron), while flesh contained the lowest concentration. Most of the analyzed samples contained lead and cadmium near or above the FAO permissible limits (FAO/WHO, 1993).

Abou-Arab *et al.* (1996) reported concentrations of some heavy metals (e.g. Pb, Cd, Cr, Cu, Zn, Mn, and Fe) in sardine and mackerel fish imported to Egypt. Lead and cadmium in sardine (11.10 and 0.86 ppm, respectively) and in mackerel (12.60 and 0.77 ppm, respectively) were found higher than the permissible limits (Pb 0.5 and Cd 0.2 ppm; FAO/WHO, 1993). Sardine seemed to be more contaminated with iron than mackerel fish.

In the Egyptian Fayoum Province, a number of private fish farms are situated around Lake Qarun. These farms culture *Tilapia* and *Mugil* fish, species only. Mansour and Sidky (2002) compared heavy metal contamination in the different kinds of fish, either those of the lake or the private farms. According to the data presented in Table 14.1, the *Mugil* sp. fish from both sources and the *Tilapia* sp. fish from the lake contained cadmium concentrations above the permissible limits proposed by FAO/WHO (1993), which is 0.2 ppm. Lead (Pb) was greatly above the permissible limit (0.5 ppm) in all of the samples analyzed. The authors added that the fish of summer season were generally more contaminated than those of the other seasons of the year.

Recently, Murtala *et al.* (2012) determined accumulation of some heavy metals (Cr, Co, Ni,

Table 14.1   Concentration (ppm) of some heavy metals in fish samples collected from Fayoum Governorate, Egypt compared to the permissible limits. After Mansour and Sidky (2002). Each value is a general mean for the concentration levels of the corresponding metal; values with asterisks (*) are above the permissible limits.

| Metal | *Tilapia* sp. | | *Mugil* sp. | | *Solea* sp. | *Penacus* sp. | Permissible limits (ppm) |
	Lake	Farm	Lake	Farm	Lake	Lake	(FAO/WHO, 1983)
Zn	8.60	17.00	16.00	18.80	25.70	11.20	40.0
Cu	1.70	3.00	5.70	4.90	0.22	4.40	30.0
Cd	0.33*	0.13	0.73*	0.32*	0.001	ND	0.20
Pb	3.70*	5.36*	6.60*	4.00*	12.20*	2.60*	0.50
Sn	0.03	0.30	0.24	0.11	1.00	ND	50.0

and Pb) in different organs of some fishes (*Hydrocynus forskahlii*, *Hyperopisus bebe occidentalis*, and *Clarias gariepinus*) collected from fishermen around Ogun Estuary, Nigeria. The accumulation of the metals in different organs showed significant differences ($p < 0.05$) except lead accumulation. However, the bioaccumulation of the heavy metals was species-related as the accumulations of the heavy metals analyzed in the sampled fishes were of the following trend: *H. forskahlii > H. bebe occidentalis > C. gariepinus*; the pattern of distribution was Ni > Cr > Co > Cd > Pb for all fish species. The levels of Ni and Cr in this study were higher than the maximum permissible limits (FAO, UNEP, FEPA and WHO) for human consumption, and that of Cd, Pb, and Co were lower.

Concentrations of heavy metals (Cr, Cd, Hg, Cu, Fe, Zn, Pb, and As) in water, sediment, and fish/invertebrate were investigated in the middle and lower reaches of the Yangtze River during 2006–2007 (Yi *et al.*, 2008, 2011). The concentrations of heavy metals were 100–10,000 times higher in the sediment than in the water. Benthic invertebrates had relatively high concentrations of heavy metals in their tissues due to their proximity to contaminated sediments. Benthic invertivore fish had moderately high concentrations of heavy metals whereas phytoplanktivore fish, such as the silver carp, accumulated the lowest concentration of heavy metals. The concentrations of Cu, Zn, and Fe were higher than Hg, Pb, Cd, Cr, and As in the tissue samples. The concentration of heavy metals was lower in the river sediments than in the lake sediments. Conversely, the concentration of heavy metals was higher in river water than in lake water. While a pollution event into a water body is often transitory, the effects of the pollutants may be long-lived due to their tendency to be absorbed in the sediments and then released into the food chain. According to the authors' results, the heavy metals were concentrated in the following order: bottom material > demersal fish and benthic fauna > middle–lower layer fish > upper–middle layer fish > water.

A number of studies have reported that concentrations of heavy metals were found highest in the sediments, intermediate in fish and lowest in the water (Enk and Mathis, 1977; Anderson *et al.*, 1978; Burrows and Whitton, 1983; Barak and Mason, 1989; Yi *et al.*, 2008, 2011). The results support the hypothesis that the sediment is the major sink for trace metal pollution and plays an important role in heavy metal uptake by fish (Luoma and Bryan, 1978; Yi *et al.*, 2001).

Studies conducted on Lake Qarun, Egypt showed that results coincide with the general trend of heavy metal distribution between water, sediment, and biota (e.g. fish) (Mansour and Messeha, 2001; Mansour and Sidky, 2002). In this connection it may be useful to compare results from an Egyptian ecosystem with results from a Chinese ecosystem (Yi *et al.*, 2011), as shown in Table 14.2 for some metals which were measured in both studies. In both cases, the accumulation of heavy metals (as total) for sediment, demersal fish and benthic fauna, and pelagic fish amounted to *c.* 7766, 298.8, and 208.3 times that of water, respectively (Yi *et al.* 2011). In Lake Qarun studies, the estimated relative accumulation index for sediment, Bouri fish (*Mugil* sp.), shrimp (*Penacus* sp.), Bulti fish (*Tilapia* sp.), and Mousa fish (*Solea* sp.) was a factor of 61, 20.3, 17.6, 11.8, and 6.6, greater than that of water, respectively (Table 14.2). It should be noted that both Bouri fish and the shrimp inhabit waters near the lake bottom, while Bulti and Mousa fish inhabit the upper layer in the lake water column.

14.2.4 Heavy metal deposition from air

The World Health Organization (WHO/Europe, 2007), Regional Office for Europe reviewed the available information on the sources, chemical properties and spatial distribution of environmental pollution with cadmium, lead, and mercury caused by long-range transboundary air pollution (LRTAP), and evaluated the potential health risks in Europe. The heavy metals cadmium, lead, and mercury are common air pollutants, being emitted mainly as a result of various industrial activities. Although the atmospheric levels are low, they contribute to the deposition and build-up in

Table 14.2 Relative accumulation of heavy metals in natural aquatic ecosystems.

Matrix	Concentration (mg kg^{-1} wet weight for fish tissue/water and dry weight for sediment)						Relative accumulation index (times that of water)
	Cu	Zn	Pb	Cd	Cr	Total	
Yi *et al.* (2011); studies conducted on the Yangtze River, China							
Water	0.0028	0.031	0.002	0.0004	0.0013	0.038	1.0
Pelagic fish	1.16	6.08	0.48	0.072	0.123	7.92	208.3
Demersal fish and benthic fauna	2.24	8.05	0.57	0.128	0.365	11.35	298.8
Sediment	45.7	135.6	37.0	0.423	76.4	295.1	7766.4
Mansour and Messeha (2001); Mansour and Sidky (2002); both studies conducted on Lake Qarun, Egypt							
Water	0.152	0.027	0.017	0.042	0.735	0.973	1.
Bolti fish	1.68	8.60	0.20	0.33	0.70	11.51	11.8
Bouri fish	5.75	10.80	1.15	0.66	1.35	19.7	20.3
Mousa fish	0.22	5.80	0.25	0.001	0.11	6.4	6.6
Shrimp	4.30	11.00	0.58	0.001	1.27	17.2	17.6
Sediment	14.75	31.80	2.60	1.77	9.20	60.1	61.8

soils. Heavy metals are persistent in the environment and are subject to bioaccumulation in food chains. Long-range transboundary air pollution is only one source of exposure to these metals but, because of their persistence and potential for global atmospheric transfer, atmospheric emissions affect even the most remote regions.

Sharma *et al.* (2009) presented data on heavy metals (Cu, Zn, Cd, and Pb) concentrations in some key Indian vegetables such as palak (*Beta vulagaris* L.; Family Chenopodiaceae), lady's finger (*Abelmoschus esculentus* L.; Family Malvaceae) and cauliflower (*Brassica oleracea*; Family: Brassicaceae) grown locally in suburban and rural areas and sold in urban open markets. It was hypothesized that atmospheric depositions in urban areas may increase the levels of heavy metals during transport and marketing, leading to significant contamination of vegetables at the market sites rather that at the production sites. Generally, contamination levels with heavy metals were higher in market sites vegetables than those from production sites, indicating deposition of heavy metals on the vegetables during transport and marketing in the more polluted urban environment. Figure 14.2 presents

a comparison between both sites with respect to contamination levels of selected vegetables with Cu, Zn, Cd, and Pb. Results also revealed that the crop production sites in the vicinity of brick kilns or near the national highway showed higher concentrations of heavy metals than those having no specific sources of emission. The higher concentration of Zn in the soil may also be ascribed to the use of Zn in fertilizers and metal-based pesticides apart from ash from brick kilns (Sharma *et al.*, 2009).

Heavy metal contamination in the street dust due to metal smelting in the industrial district of Huludao city, China was investigated by Zheng *et al.* (2010). The maximum Hg, Pb, Cd, Zn, and Cu contents in the street dust were found to be 5.212, 3903, 726.2, 79,869, and 1532 mg kg^{-1}, 141, 181, 6724, 1257, and 77.4 times as high as the background values in soil. The biggest contribution to street dust is atmospheric deposition due to metal smelting, but traffic density makes a small contribution to heavy metal contamination. According to the calculation of hazard index (HI), the ingestion of dust particles by children and adults in Huludao city appears to be the route of exposure to street dust that results in a
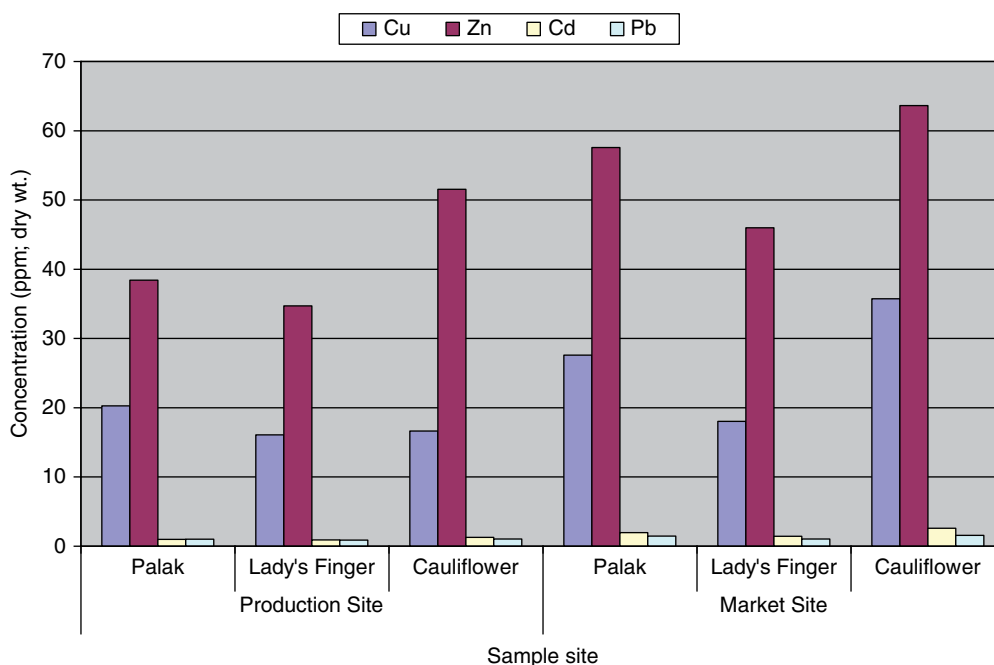
Figure 14.2 Mean concentrations (µg g^{-1} dry weight) of some heavy metals in selected vegetables from production and market sites of Varanasi, India. Vegetables: palak (*Beta vulagaris*); lady's finger (*Abelmoschus esculentus*); cauliflower (*Brassica oleracea*). Adapted from Sharma *et al.* (2009). For color details, see color plates section.

higher risk for heavy metals, followed by dermal contact. The inhalation of resuspended particles through the mouth and nose is almost negligible. The inhalation of Hg vapor as the fourth exposure pathway to street dust accounts for the main exposure. The hazard index for lead (HI > 1) indicates that children are exposed to a potential health risk; for cadmium HI ~ 1, implying a probably health risk.

14.3 Multiple environmental factors affecting accumulation of heavy metals in food and impact on human health

Concentrations of Cd, Ni, and Pb in vegetables (e.g. spinach, radish, and tomato) grown under the influence of atmospheric deposition and wastewater irrigation in the urban environment

of Varanasi, India were reported by Pandey *et al.* (2012). The estimated levels exceeded the standard safe limits. The study indicated that the atmospheric depositions as well as wastewater irrigation have significantly elevated the levels of heavy metals in dietary vegetables, presenting a significant threat to the health of users. Figure 14.3 depicts the contribution of each contamination source to the daily intake of selected heavy metals by spinach, radish, and tomato, based on the maximum values given by the authors (Pandey *et al.*, 2012).

The concentrations of heavy metals such as Pb, Cd, Cu, and Zn have been estimated in air particulates, water, and food samples collected from different suburbs in Bombay during 1991–1994 (Tripathi *et al.*, 1997). The concentrations of these metals were translated into intake rates through inhalation and ingestion pathways. Results indicated that the highest concentrations of Pb and Cu are in pulses (green gram), Cd in
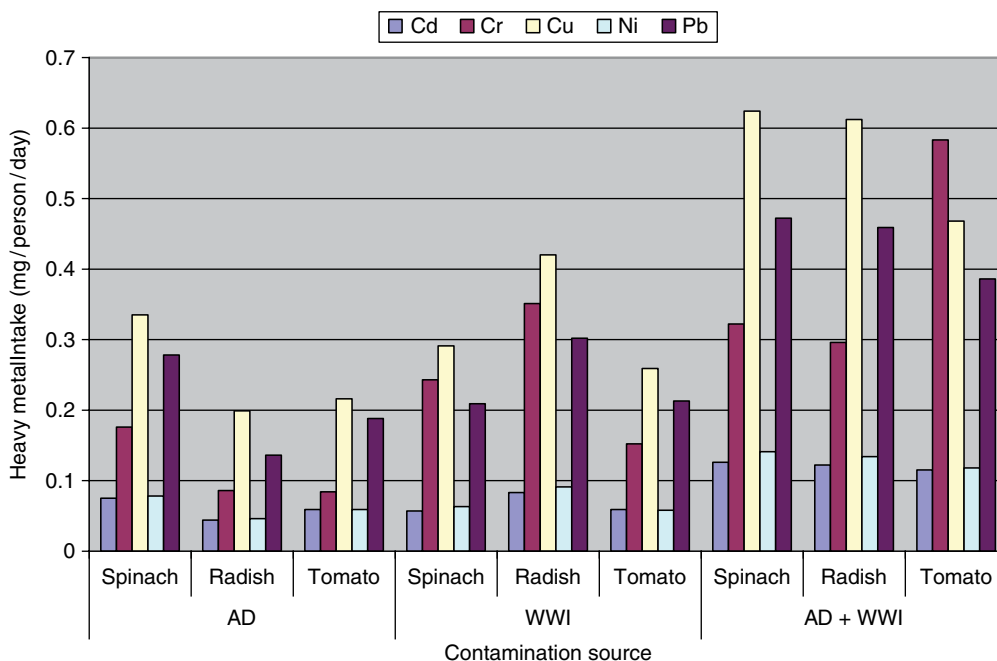
Figure 14.3   Daily intake of heavy metals (mg/person/day) in different vegetables as influenced by atmospheric deposition (AD), waste water irrigation (WWI) and a combination of these (AD+WWI). Adapted from Pandey *et al.* (2012). For color details, see color plates section.

leafy vegetables (amaranth), and Zn in meat. Root vegetables and fruits contained a lower concentration of these metals. Total intakes of Zn, Cu, Pb, and Cd through air, water, and food were 10,500 µg day⁻¹, 1500 µg day⁻¹, 30 µg day⁻¹ and 4.3 µg day⁻¹, respectively. Although the major contribution to the daily intake is the ingestion route, eventual uptake in the body stream is achieved through inhalation for Pb (41%) and Cd (16%) and ingestion for Cu (98.8%) and Zn (99.6%). The total intake of these elements through the duplicate diet study is 9500 µg day⁻¹ for Zn, 1770 µg day⁻¹ for Cu, 27 µg day⁻¹ for Pb and 2.5 µg day⁻¹ for Cd, respectively. The daily intake of these metals by the population of Bombay is well below the recommended dietary values.

Salem *et al.* (2000) reported that there is a strong relationship between certain heavy metals and chronic diseases such as renal failure, liver cirrhosis, hair loss, and chronic anemia identified

in some locations in Greater Cairo, Egypt. These diseases are apparently related to drinking water contaminated with heavy metals such as Pb, Cd, Cu, Mo, Ni, and Cr. Renal failure is related to drinking water contaminated with lead and cadmium, liver cirrhosis to copper and molybdenum, hair loss to nickel and chromium, and chronic anemia to copper and cadmium. Studies of these diseases suggest that abnormal incidences in specific areas is related to industrial wastes and agriculture activities that have released hazardous and toxic materials in the groundwater and thereby led to the contamination of drinking water in these areas.

On a global scale, Fewtrell *et al.* (2004) assessed blood lead (B-Pb) concentration levels in different parts of the world. It appeared that the highest B-Pb levels occur in South and Central America, the Middle East, parts of Eastern Europe, and the countries of the former USSR (Figure 14.4). The survey results revealed that about 25% or
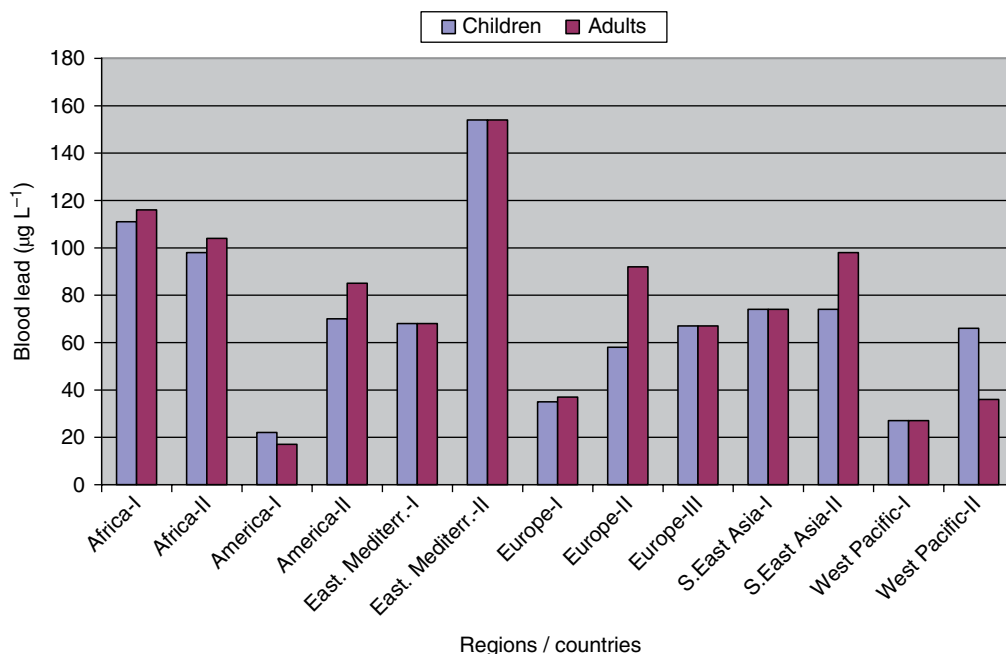
Figure 14.4   Concentration of blood-Pb in urban children and adults in different areas. Adapted from Fewtrell *et al.* (2004). Countries involved in each region: Africa-I: Nigeria; Africa-II: South Africa; America-I: Canada, United States; America-II: Argentina, Brazil, Chile, Jamaica, Mexico, Uruguay, Venezuela, Ecuador, Nicaragua, Peru; Eastern Mediterranean-I: Saudi Arabia; Eastern Mediterranean-II: Egypt, Morocco, Pakistan; Europe-I: Denmark, France, Germany, Greece, Israel, Sweden; Europe-II: Turkey, Yugoslavia; Europe-III: Hungary, Russian Federation. South-East Asia-I: Indonesia, Thailand; South-East Asia-II: Bangladesh, India; Western Pacific-I: Australia, Japan, New Zealand, Singapore; Western Pacific-II: China, Philippines, Republic of Korea. Source: Fewtrell Prüss-Üstün *et al.*, 2004. Reproduced with permission of Elsevier. For color details, see color plates section.

more of the children in these areas have B-Pb levels above $100 \mu g L^{-1}$. In Australia, North America, and Western Europe, the corresponding proportion of children was less than 10%.

Many researchers have used the pollution index of soils and sludges to identify element contamination resulting in the increased overall element toxicity (Chon *et al.*, 1997). Although the results differ between locations and between researchers, the basic concept remains the same. Pollution index is calculated by the average ratio of the metal concentration in the sample to the tolerable/permissible levels of soil for the plant growth suggested by Kloke *et al.* (1984).

An example of the potential exposure of a population to a heavy metal such as cadmium in some countries is demonstrated by Zhang *et al.* (1997).

The authors compared cadmium intake and uptake via respiratory and dietary routes in China and Japan. On average, the cadmium concentration in air was $7.3 \, ng \, m^{-3}$ in China, the daily exposure via the respiratory tract was calculated to be $0.11 \, \mu g$ and the uptake (50% absorption) was $0.05 \, \mu g$. The daily intake via food was reported to be $9.9 \, \mu g$ and the uptake (7.5% absorption) was $0.74 \, \mu g$ day^{-1}. In Japan, uptakes via the respiratory and food routes were calculated to be 0.07 and $2.41 \, \mu g$ day^{-1}, respectively. The uptake via food was estimated to amount to 93.7% in China and 97.2% in Japan. In the Czech Republic, the daily intakes via the respiratory route, water, and food were estimated to be $0.01 \, \mu g \, day^{-1}$, $0.17 \, \mu g \, day^{-1}$ and $18.2 \, \mu g \, day^{-1}$ (0.05%, 0.92%, and 99.3%), respectively (Kliment, 1996).

In Canada, the estimated daily cadmium intakes in adults over 20 years (assumed to weigh 70 kg, to breath 23 m³ of air, drink 0.4 L of water, ingest 20 mg of soil daily, and to smoke 20 cigarettes/day) were as follows: from air the intake was 0.33–1.3 ng kg⁻¹ bw day⁻¹; from drinking water it was <0.057–0.51 ng kg⁻¹ bw day⁻¹; from food it was 210 ng kg⁻¹ bw day⁻¹; from soil it was 0.16–0.33 ng kg⁻¹ bw day⁻¹; and from cigarettes it was 53 ng kg⁻¹ bw day⁻¹ (Newhook *et al.*, 1994).

At a cadmium concentration of 1 mg/kg soil, the intake of cadmium from ingestion of soil would be approximately 0.05–0.2 μg/day, assuming a soil-ingestion rate of 0.05–0.2 g/day for adults (Choudhury *et al.*, 2001). The data presented above indicate that food is the main source of cadmium exposure in the general population, being responsible for more than 90% of the total intake in non-smokers.

Beyond the problem of dietary exposure to heavy metals, exposure through children's toys may represent a health risk. In this respect, Sindiku and Osibanjo (2011) determined the level of lead, cadmium, chromium, and nickel in the plastic components of 51 toys manufactured from different countries and imported in Nigeria. The results obtained showed that lead, cadmium, chromium, and nickel were high and within the range 28.5–12,600 mg kg⁻¹ Pb, 0.15–9.55 mg kg⁻¹ Cd, 1.30–394.50 mg kg⁻¹ Cr, and 5.9–1911 mg kg⁻¹ Ni. A comparison of the mean concentration of these metals in the toy samples analyzed showed the following pattern: Pb > Ni > Cr > Cd. Compared to the total threshold limit concentration (TTLC) of 90, 75, and 60 mg kg⁻¹ for lead, cadmium, and chromium, respectively, Consumer Product Safety Commission, USA, Bureau of Indian Standard and Thailand Industrial Standard for Toys suggested that these toys are hazardous and therefore not safe for children's use. This underscores the need for urgent national policy and resolution control on the removal of heavy metals, especially lead, from children's toys. Based on the data provided by the authors, a total of 43 types of toys were manufactured in China, 4 in the USA, 2 in the UK and 1 from each of Vietnam and Romania (Sindiku and Osibanjo, 2011).

14.4 Comparative levels of heavy metals in vegetables and fruits from different countries

Monitoring and assessment of heavy metal concentrations in vegetables from market sites have been carried out in some developed (Jorhem and Sundstroem, 1993; Milacic and Kralj, 2003), and developing countries (Tripathi *et al.*, 1997; Abou-Arab *et al.*, 1999; Agrawal, 2003; Parveen *et al.*, 2003; Dogheim *et al.*, 2004; Marshall, 2004; Jassir *et al.*, 2005; Radwan and Salama, 2006; Sharma *et al.*, 2008, 2009; Mansour *et al.*, 2009a,2009b). From such reports and others, we quote data which may shed light on the problem of heavy metal food contamination in different countries.

Several investigators compared contamination levels of heavy metals at different locations in various countries. The data used in these comparisons are derived from experimental samples produced under different environmental and practical conditions and analyzed by different techniques and methodologies; however, they may provide a provisional view of the contamination status. In this context, Table 14.3 presents data from the literature, focusing on selected heavy metals (e.g., Pb, Cd, Cu, Zn) on a number of vegetables and fruits from some countries.

Comparing concentration levels of a specific heavy metal in a vegetable based on data published in the literature may yield 'dissimilar' values to a great extent. This is highly acceptable since farming, production, and marketing conditions, which might be extremely different, will be taken into account. On the other hand, obtaining 'equally similar' values does not mean complete uniformity of all farming practices and conditions between the many countries involved in such comparison.

From Table 14.3, potato as a worldwide food commodity was found to contain 0.02 and 0.03 mg kg⁻¹ cadmium according to Radwan and Salama (2006) and Mansour *et al.* (2009b), respectively. The values are roughly similar, however sampling times were different. Cadmium values of 0.022, 0.08, 0.031, 0.015, and 0.337 mg kg⁻¹ were

Table 14.3 Levels of Pb, Cd, Cu, and Zn in selected fruits and vegetables: comparison between some countries.

Country	Metal	Apple	Orange	Banana	Spinach	Lettuce	Eggplant	Cucumber	Potatoes	Reference
					Concentration (mg kg⁻¹) in foodstuff					
Egypt	Pb	0.19	0.15	0.05	0.34	0.58	0.21	0.19	0.08	Radwan and Salama (2006)
		-	-	-	0.56	0.07	-	-	-	Dogheim et al. (2004)
		-	-	-	-	-	-	0.26	-	Mansour et al. (2009a)
		-	-	-	-	-	-	-	0.34	Mansour et al. (2009b)
	Cd	0.05	0.04	0.02	0.11	0.07	0.02	0.15	0.02	Radwan and Salama (2006)
		-	-	-	0.03	0.01	-	-	-	Dogheim et al. (2004)
		-	-	-	-	-	-	0.07	-	Mansour et al. (2009a)
		-	-	-	-	-	-	-	0.03	Mansour et al. (2009b)
	Cu	1.47	1.27	2.51	4.48	1.97	1.41	5.69	0.83	Radwan and Salama (2006)
		-	-	-	1.18	0.92	-	-	-	Dogheim et al. (2004)
		-	-	-	-	-	-	0.60	-	Mansour et al. (2009a)
		-	-	-	-	-	-	-	0.77	Mansour et al. (2009b)
	Zn	1.36	2.38	5.59	20.89	9.76	11.48	19.94	7.16	Radwan and Salama (2006)
		-	-	-	-	-	-	1.36	-	Dogheim et al. (2004)
		-	-	-	-	-	-	-	2.33	Mansour et al. (2009b)
Greece	Cd	0.0003	0.0009	0.001	0.053	0.052	0.032	0.0002	0.022	Karavoltsos et al. (2002)
Pakistan	Pb	0.76	-	-	-	-	1.30	1.72	0.16	Parveen et al. (2003)
	Cd	0.14	-	-	-	-	0.31	0.36	0.08	Parveen et al. (2003)
	Cu	0.50	-	-	-	-	3.14	4.45	0.10	Parveen et al. (2003)
	Zn	2.05	-	-	-	-	3.52	3.22	ND	Parveen et al. (2003)
Tanzania	Pb	-	-	-	0.30–0.59	0.36–0.38	-	-	-	Bahemuka and Mubofu (1999)
	Cd	-	-	-	0.03–0.06	0.03–0.04	-	-	-	Bahemuka and Mubofu (1999)
	Cu	-	-	-	0.72–1.37	0.25–0.58	-	-	-	Bahemuka and Mubofu (1999)
	Zn	-	-	-	4.08–4.81	1.48–1.59	-	-	-	Bahemuka and Mubofu (1999)
Nigeria	Cu	0.25	2.13	0.95	-	0.72	5.47	-	0.72	Onianwa et al. (2001)
	Zn	0.16	2.20	1.50	-	2.30	9.67	-	3.00	Onianwa et al. (2001)

(Continued)

Table 14.3 (Continued)

Country	Metal	Apple	Orange	Banana	Spinach	Lettuce	Eggplant	Cucumber	Potatoes	Reference
					Concentration (mg kg^{-1}) in foodstuff					
USA	Pb	-	-	-	-	0.013	-	-	0.009	Radwan and Salama (2006)
	Cd	-	-	-	-	0.26	-	-	0.031	Radwan and Salama (2006)
	Cu	-	-	-	-	-	-	-	0.64	Radwan and Salama (2006)
	Zn	-	-	-	-	-	-	-	2.10	Radwan and Salama (2006)
India	Pb	-	-	-	3.10	-	3.0	-	-	Chary et al. (2008)
	Cu	-	-	-	0.09	-	0.70	-	-	Chary et al. (2008)
	Zn	-	-	-	10.0	-	4.50	-	-	Chary et al. (2008)
China	Pb	-	-	-	0.09	0.11	-	-	-	Khan et al. (2008)
					0.031	-	0.06	0.035	0.067	Song et al. (2009)
	Cd	-	-	-	0.81	1.47	-	-	-	Khan et al. (2008)
					0.018	-	0.015	0.003	0.015	Song et al. (2009)
	Cu	-	-	-	0.45	0.48	-	-	-	Khan et al. (2008)
					0.70	-	0.77	0.45	1.03	Song et al. (2009)
	Zn	-	-	-	0.39	0.41	-	-	-	Khan et al. (2008)
					2.99	-	1.70	1.64	3.77	Song et al. (2009)
Lebanon	Pb	-	-	-	0.74	0.625	0.04	0.056	0.076	Al-Chaarani et al. (2009)
	Cd	-	-	-	1.25	0.629	0.324	0.297	0.337	Al-Chaarani et al. (2009)

recorded in Greece (Karavoltsos *et al.*, 2002), Pakistan (Parveen *et al.*, 2003), USA (Radwan and Salama, 2006), China (Song *et al.*, 2009), and Lebanon (Al-Chaarani *et al.*, 2009), respectively. Only the result from Lebanon was extremely higher than the other values from different countries; however, the estimation of Cd concentration was calculated on the dry weight basis of potato tubers in contrast to the other investigations.

Concentration levels for Pb in lettuce (Table 14.3) yielded the following very different results: $0.58 \, mg \, kg^{-1}$ (Radwan and Salama, 2006) and $0.07 \, mg \, kg^{-1}$ (Dogheim *et al.*, 2004) in Egypt; *c*. $0.37 \, mg \, kg^{-1}$ (Bahemuka and Mubofu, 1999) in Tanzania; $0.013 \, mg \, kg^{-1}$ (Radwan and Salama, 2006) in the USA; $0.11 \, mg \, kg^{-1}$ (Khan *et al.*, 2008) in China; and $0.625 \, mg \, kg^{-1}$ (Al-Chaarani *et al.*, 2009) in Lebanon. Table 14.3 also contains contamination data reported by other investigators (Onianwa *et al.*, 2001; Chary *et al.*, 2008; Mansour *et al.*, 2009a).

14.5   Removal of heavy metal contamination

Removal of heavy metal contaminants includes direct removal from food commodities (e.g. vegetables and fruits) and indirect removal from irrigation water and soil where the plant crop is cultivated. Such indirect processes may lead to a reduction of plant uptake and accumulation of heavy metals in the fruits.

14.5.1   Vegetable/fruit decontamination

Heavy metals may be present either as a deposit on the surface of vegetables, or may be taken up by the crop roots and incorporated into the edible part of plant tissues. Heavy metals deposited on the surface can often be eliminated simply by washing prior to consumption and peeling, whereas bio-accumulated metals are difficult to remove and are of major concern.

Al-Chaarani *et al.* (2009) estimated the efficiency of thorough washing of certain vegetable samples with water before analysis; the concentrations of toxic metals (Pb, Cd, Cr, As) are reduced to some extent. Washing of cauliflower did not affect Pb levels, but significantly decreased the concentration of Cd. On the other hand, Cd in cucumber showed little removal, while Cr was greatly reduced by washing.

14.5.2   Wastewater treatment

Many toxic heavy metals have been discharged into the environment as industrial wastes, causing serious soil and water pollution (Lin and Juang, 2002). Pb^{+2}, Cu^{+2}, Fe^{+3}, and Cr^{+3} are especially common metals that tend to accumulate in organisms, causing numerous diseases and disorders (Inglezakis *et al.*, 2003). They are also common groundwater contaminants at industrial and military installations. Numerous processes exist for removing dissolved heavy metals in wastewater including ion exchange, precipitation, phytoextraction, ultrafiltration, reverse osmosis, and electrodialysis (Applegate, 1984; Sengupta and Clifford, 1986; Geselbarcht, 1996; Schnoor, 1997). The use of alternative low-cost materials as potential sorbents for the removal of heavy metals has been emphasized recently.

Activated carbon adsorption is considered to be a particularly competitive and effective process for the removal of heavy metals at trace quantities (Huang and Blankenship, 1984); however, the use of activated carbon is not suitable in developing countries due to the high costs associated with production and regeneration of spent carbon (Panday *et al.*, 1985). Various treatment processes are available, among which ion exchange is considered to be cost-effective if low-cost ion exchangers such as zeolites are used (Bailey *et al.*, 1999). Natural zeolites hold great potential to remove cationic heavy metal species from industrial wastewater (Erdem *et al.*, 2004).

14.5.3   Plant- and animal-derived materials

A starch derivative has been developed as an effective metal scavenger at USDA in Peoria, Illinois, USA (Wing, 1974; Wing and Doane, 1976).

The starch was substituted with xanthate esters, which can form chelates with various heavy metal ions in the aqueous medium. As xanthation is a relatively simple process and starch itself is a cheap biopolymer, the derivative can be produced at an economical cost. However, xanthate degrades slowly and generates a sulfide off-flavor during the storage and application (Wing and Doane, 1976).

Corn starch was cross-linked and carboxymethylated at a relatively low degree of substitution (DS), and its removal capacity for various divalent metal ions from their aqueous solutions was investigated by Kim and Lim (1999). Lead, cadmium, and mercury ions in water were almost completely removed when 1% starch (DS 0.081, pH 6.0) was used. Under the same conditions, copper concentration was reduced from 203 to 71 ppm. Starch could be recovered by washing the metal ions from the complex with weak acid (pH 2.0), although the metal-binding activity of the starch was slightly reduced by this process.

Ekebafe et al. (2012) used cassava starch hydrogel for removing heavy metals such as Pb, Cu, and Ni from aqueous media. The authors reported that the use of native cassava starch grafted poly(acrylonitrile) for heavy metal ions removal is technically feasible, eco-friendly, and highly efficient. Being composed of natural polysaccharides, it also helps in reduction of environmental pollution and safe disposal of heavy metals, being biodegradable. This sorbent is a good candidate for sorption of not only heavy metal ions but also other organics in waste water stream. Grafting enhances the uptake of metal ions from aqueous media and the extent of uptake is influenced by the sizes of the ions. These results are of interest to the development of hydrogel-based technologies for water purification and metal ions separation and enrichment.

According to Li et al. (2004), silicate colloids with an average diameter of 100 nm were prepared by the hydrolysis of tetraethoxysilane (TES), NH_4OH (30%) and then modified by (3-mercaptopropyl) trimethoxysilane (APS). The colloids can adsorb heavy metals such as Pb and Cr in effluent and, after adsorption, the colloids can be separated by coagulation of aluminum sulfate. The removal of heavy metals was found to reach up to 99%.

According to An et al. (2001), total crab landings have reported a steady increase over the 1990–1997 period from 8.87 to 10^8 tons in 1990 to almost 1.2–10^9 tons in 1997 (FAO, http://www.fao.org/). Most crab products are processed into canned crabmeat or frozen goods. The crab shells can be obtained cheaply as byproducts of the process. An et al. (2001) studied the ability of raw crab shell to remove heavy metals (Pb, Cd, Cu, Cr) from aqueous solution by comparing with that of cation exchange resin, zeolite, granular activated carbon, and powdered activated carbon. The ability to remove heavy metals was assessed by the heavy metal removal capacity, removal rate, and removal efficiency. The orders of heavy metal removal capacity and initial heavy metal removal rate were found as crab shell > cation exchange resin > zeolite > powdered activated carbon ≥ granular activated carbon. Crab shell is therefore satisfactory as a good biosorbent for the heavy metal removal. The study indicated that the removal of these heavy metals is selective, with Pb and Cr being removed in preference to Cd and Cu. The sorption equilibrium of heavy metal ions on sorbents was modeled on the applications of Langmuir and Freundlich.

14.5.4 Soil remediation

Soil contamination by heavy metals is consequently the most critical environmental problem as it poses significant impacts to human health as well as ecosystems. Contaminants are able to infiltrate deep into the layer of underground waters and pollute the groundwater as well as the surface water. Heavy metals in the soil subsequently enter the human food web through plants and constitute a risk to the ecosystem as they tend to bioaccumulate and can be transferred from one food chain to another. Heavy metals are discovered in various food chains where the results are usually detrimental to microorganisms, plants, animals, and humans alike (Abioye, 2011).

Many techniques of remediation of contaminated soil have been developed such as physical degradation, chemical degradation, and photodegradation. However, these methods have some drawbacks in completely remediating hydrocarbon-contaminated soil. Some of these methods leave behind daughter compounds which are more toxic to the environment than the parent compounds. Biological treatment offers the best environmentally friendly method for remediating hydrocarbon and heavy-metal-contaminated soil because it utilizes the capability of the indigenous microorganisms in the soil environment to break down the hydrocarbons and heavy metals into innocuous substances (Abioye, 2011).

14.5.5 Soil bioremediation

Biological remediation, a process defined as the use of microorganisms or plants to detoxify or remove organic and inorganic xenobiotic compounds from the environment is a remediation option that provides a green technology solution to the problem of environmental degradation. This process relies upon microbial enzymatic activities to transform or degrade the contaminants from the environment (Philp and Atlas, 2005). It offers a cost-effective remediation technique compared to other remediation methods, because it is a natural process and does not usually produce toxic byproducts. It also provides a permanent solution as a result of complete mineralization of the contaminants in the environment (Perelo, 2010). Advantages of biological remediation compared to other treatment methods include (Okoh and Trejo-Hernandez, 2006): (1) destruction rather than transfer of the contaminants to another medium; (2) minimal exposure of workers to the contaminants; (3) long-term protection of public health; and (4) possible reduction in the duration of the remediation process.

Many genera of microbes such as *Bacillus*, *Enterobacter*, *Escherichia*, *Pseudomonas* and also some yeasts and moulds help in bioremediation of metal and chromium-contaminated soil and water by bio-absorption and bioaccumulation of chromium (Kotas and Stasicka, 2000). Heavy metal removal by the bacteria *Pseudomonas* was attributed to the cellular growth of these organisms (Ray and Ray, 2009). In their study, Damodaran *et al.* (2011) used *Saccharomyces cerevisiae* for the removal of heavy metals such as lead and cadmium from contaminated soil. The tolerance of *Saccharomyces cerevisiae* to the metals was found to be up to 250 ppm for Pb^{2+} and 500 ppm for Cd^{2+}. The parameters affecting the biosorption of heavy metals such as time, metal concentration, and biomass concentrations have been investigated. The results revealed that biosorption of about 67–82 % of Pd^{2+} and 73–79 % of Cd^{2+} was attained within 30 days. The time taken for maximum sorption of Pb^{2+} and Cd^{2+} was 30 days for soil containing 100 and 300 ppm of Pb^{2+} and Cd^{2+}, respectively. Biosorption rate are higher when the cells are in stationary phase. The biosorption and the growth of the microorganism in aerated soil were found to be comparable to that of non-aerated soil.

14.5.6 Soil remediation by metal phytoextraction

Some plants able to take up heavy metals from contaminated soils offer the possibility to clean up sites contaminated with heavy metals. Plants act as a solar-driven pump, which can extract and concentrate heavy metals from the environment. Since most of the metal hyper-accumulating wild plants only produce very low biomass and most of plants producing high biomass accumulate only moderate amounts of metals, current research is mainly focused on overcoming this deficiency to optimize metal phytoextraction. Sunflower, tobacco, and maize are examples of plants used for phytoextraction of some heavy metals (e.g. Zn, Cd, Cu) from the soil (Kayser *et al.*, 2000).

Moreno-Jiménez *et al.* (2009) analyzed 25 vascular plant species (3 ferns and 22 flowering plants) grown in soils surrounding an abandoned mine in NW Madrid (Spain) in order to identify exceptional characteristics that would be interesting for soil phytoremediation and/or reclamation. Many of these plants survive in contact with relatively high levels of heavy metals in the

soils. The results showed that high Cd and Zn concentrations have been found in the aerial parts of *Hypericum perforatum* (Cd), *Salix atrocinerea* (Cd, Zn) and *Digitalis thapsi* (Cd, Zn). The phytoremediation ability of *S. atrocinerea* for Cd and Zn was estimated, obtaining intervals of time that could be considered suitable for the phytoextraction of polluted soils.

Metal concentrations were measured in plants growing on heavily contaminated tailings from a mine active since about 1800 in San Luis Potosí (Mexico) (Franco-Hernández *et al.*, 2010). The plants *Viguiera dentata* (Cav.) Spreng., *Parthenium bipinnatifidum* (Ort.) Rollins, *Flaveria angustifolia* (Cav.) Pers., *F. trinervia* (Spreng.) C. Mohr, and *Sporobolus indicus* (L.) R. Br. were found tolerant to high As, Cu, Pb, and Zn concentrations. Of those, *S. indicus* excluded heavy metals from its shoots, while *P. bipinnatifidum* and *F. angustifolia* accumulated them. *V. dentata* and *P. bipinnatifidum* were accumulators of As, but not hyper-accumulators. It was found that *V. dentata*, *P. bipinnatifidum*, *F. angustifolia*, *F. trinervia*, and *S. indicus* could be used to vegetate soils contaminated with As, Cu, Pb, and Zn. *Ambrosia artemisifolia* could be used to remediate soils contaminated with Zn, *S. amplexicaulis* for soils with Cu and *F. angustifolia* and *F. trinervia* for soils with As, as they have a strong capacity to accumulate those metals.

14.6   Prevention and reduction of metal contamination in food

Food passes through several stages in the long and sophisticated food chain processing (from farm to fork) before being consumed. Each stage can cause morbidity and mortality, and also affect food industry. Food is a vulnerable media for contamination by thousands of biological, chemical, and physical agents and radionuclear materials. Such contamination may occur intentionally or unintentionally by the following routes.

1. *Residual chemicals*: contaminants already present within or on a foodstuff resulting from previous exposure of ingredients or raw materials during food production, processing, or packaging.
2. *Applied chemicals*: applied intentionally to foodstuffs and intended to be retained, e.g. food additives and food preservatives.
3. *Accidental chemicals*: applied accidentally or generated unintentionally. Examples include impurities in applied chemicals and migratory species from materials such as packaging.
4. *Background chemicals*: ubiquitous environmental contaminants that may enter the food chain at almost any stage of food production, distribution and handling, such as polycyclic aromatic hydrocarbons and polychlorinated biphenyls, dibenzo-p-dioxins and dibenzofurans.

It has long been recognized that heavy metals are among the major contaminants of food supply and may be considered as the most important problem to our environment. They can contaminate food via all the above-mentioned pathways. For instance, heavy metal contamination may occur in the field due to irrigation with contaminated water, the addition of fertilizers and metal-based pesticides, industrial emissions, transportation, harvesting process, storage, and/or sale. It is well known that plants take up metals by absorbing them from contaminated soils as well as from deposits on parts of the plants exposed to the air from polluted environments. Unfortunately, they cannot be degraded or destroyed but tend to bio-accumulate in the food web, ending up in the human body. A continuous surveillance system of contaminants content in food is therefore crucial for consumer protection and facilitates international trade. Vegetable decontamination, irrigation and wastewater treatment, soil microbial bioremediation, and metal phytoextraction are among the methods of minimizing heavy metal contamination in such food commodities.

14.7 Recent technologies for removal of heavy metal contaminants

Carlos *et al.* (2013) demonstrated the application of magnetite nanoparticles for heavy metal removal from wastewater. Because access to safe drinking water is key to protecting public health, clean water has become a basic need of all properly functioning societies. Contamination of water with toxic metal ions (Hg(II), Pb(II), Cr(III), Cr(VI), Ni(II), Co(II), Cu(II), Cd(II), Ag(I), As(V) and As(III)) is becoming a severe environmental and public health problem. In order to achieve environmental detoxification, various techniques such as adsorption, precipitation, ion exchange, reverse osmosis, electrochemical treatments, membrane filtration, evaporation, flotation, oxidation, and biosorption processes are extensively used (Ivanov *et al.*, 2004; Chen *et al.*, 2009, 2010). The development of novel and cost-effective nanomaterials for environmental remediation, pollution detection, and other applications has attracted considerable attention. Recent advances suggest that many of the issues involving water quality could be resolved or greatly ameliorated using nanoparticles, nanofiltration, or other products resulting from the development of nanotechnology (Schulte and Dutta, 2005; Auffan *et al.*, 2007).

The synthesis of magnetite nanoparticles has been intensively developed not only for its fundamental scientific interest but also for many technological applications such as magnetic resonance imaging, ferrofluids for audio speakers, magnetic targeted drug delivery, and magnetic recording media. Those interested in results related to the application of magnetite nanomaterials for the removal of heavy metals and metalloids from water, mainly copper, chromium, mercury, and arsenic can refer to Carlos *et al.* (2013). Cundy *et al.* (2008) also reported that a range of environmental clean-up technologies have been proposed or developed which utilize iron chemistry to remediate contaminated land and surface and subsurface waters, e.g. the use of injected zero-valent iron nanoparticles to remediate organic contaminant plumes or the generation of iron oxyhydroxide-based substrates for arsenic removal from contaminated waters.

As a matter of fact, there may not be an optimal system for all food businesses at all stages along the sophisticated food chain but current hazard analysis and critical control point (HACCP) approaches have clear benefits (Sekheta *et al.*, 2006). The globalization of food trade is considered to be able to contribute to more widespread dissemination of hazards. From another perspective, however, globalization may increase the sources of food available to affluent urban populations, which increases their '"food entitlements" and thereby decreases their vulnerability to shock in the food supply' (Kleter *et al.*, 2009). Prevention and response are the two major strategies for countering the threat of food contamination (WHO, 2008), which is everyone's responsibility all over the world. Cooperation at regional and international levels is crucial.

14.8 Conclusion

Heavy metals can find their way easily to the human body through the food web causing detrimental health effects when they accumulate in significant amounts. Heavy metals contaminating vegetables and fruits can be eliminated by washing and peeling prior to consumption. Wastewater and polluted soils can be remediated by several chemical and biological processes. The introduction of recent technologies such as magnetic nanoparticles has yielded promising results. Contamination of food with heavy metals is a problem of global concern, requiring prevention/reduction strategies at global scale.

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15
Radionuclides in Food: Past, Present and Future

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Summary

Radionuclides, whether natural or formed as a result of human interventions, can easily become bio-concentrated along the human food chain. Monitoring and estimating the levels of radionuclides in various food products as well as their effective dose delivered on ingestion are important from a food safety point of view. Transfer and bio-accumulation of radionuclides along the terrestrial and aquatic food chain has been extensively studied for more than four to five decades. The human population is generally exposed to radioactivity from radionuclides due to their capacity to dissolve readily in water, especially those of uranium and thorium series, and thus enter the food chain (foodstuffs) via water or soil modes, respectively. In fact, radionuclides such as strontium (^{90}Sr) and caesium (^{137}Cs) can also enter the geo-chemical cycle and human food chain via organic debris of the dead plants. In the present chapter, we highlight and discuss various issues such as the presence of natural and artificial radionuclides detected in nature and present along the human food chain with special emphasis laid on radioactive uranium, thorium and radium series, covering radioactive polonium, lead, strontium and caesium, including their effects on human health. In addition, we have attempted to provide vital information on various methods employed for the detection as well as calculations, which are commonly employed to determine radionuclides in a foodstuff. Finally, we have tried to identify various gaps with regard to the ongoing research in this field, which are all expected to benefit health-conscious consumers.

15.1   Introduction

Ensuring food safety is the responsibility of not only the local governing bodies but is also the liability of non-governmental organizations (NGOs) as well as the local residing population worldwide. Monitoring and estimating the presence of toxic compounds or elements (natural or introduced) along the food processing chain or those present in a foodstuff is imperative from the point of view of consumers health. Exposure to a harmful toxicant can either have an immediate effect or can procude long-term adverse effects, which can be fatal in certain instances. Estimating dietary exposure to toxic contaminants in local or imported foods, and identifying the major contributors to this, is an integral part of food safety assessment procedure (including that of hazard identification and characterization). Some of the toxic elements accumulated naturally in a foodstuff (origin of a plant or animal source, or their products such as milk, meat, etc.) include radionuclides, heavy metals, dioxins, antinutrients, pesticides and fertilizer residues. Evidence shows that the deposition of radiotoxic elements such as uranium, thorium, radium and their series in vital organs or in body fluids (even at trace levels) can lead to a wide range of physiological disorders (Akhterb *et al*., 2003). Accumulation of toxic compounds can be at any stage along the food production or processing chain, so monitoring this is an important aspect of food safety and hazard analysis. In fact, the average daily intake of ^{238}U, ^{226}Ra, ^{232}Th and ^{228}Ra can vary between locations worldwide (Amaral *et al*., 1992; Hongda 1997; Ghiassi-Nejad *et al*., 2003; Vega *et al*., 2012; Sathyapriya *et al*., 2012).

In the present chapter, we discuss various radionuclides (commonly referred to as nuclides, radioactive elements or radioactive isotopes) detected in various foodstuffs, their effects on human health and the need for ensuring safety of food from radioactive elements. These details are expected to benefit consumers in providing knowledge on the safety aspects of the foods they consume, which may possibly be contaminated by radioactive elements, as well as ensuring the general population are prepared for cross-contaminants that might occur during a possible radiation accident in the future.

15.2   Radionuclides in nature

In nature, nearly 60 abundantly distributed radionuclides have been detected. Radionuclides are usually encountered in aquatic water bodies (e.g. in ocean, sea or lakes) or in terrestrial strata (e.g. soil or rocks), which can be easily bio-concentrated into the food chain (Skwarzec, 1988; Carvalho, 1995; Bhat *et al*., 2005). The origin of radionuclides has been differentiated into those which were formed before the creation of Earth (primordial), those produced from cosmic ray interactions (cosmogenic) and those produced due to human interventions (artificial radionuclides) (see Figure 15.1). According to Ziqiang *et al*. (1988), radionuclides are formed by nucleo-synthesis processes in stars which are characterized by their half-lives comparable to the age of the Earth.

Primordial radionuclides have been detected in rocks (monozite or igneous rocks) and are dominated by: uranium (235, 238) with a half-life of 704 million years and 4.47 billion years, respectively; radium 226 with a half year of 1601 years; radium 228 with a half-life of 5.75 years; thorium 232 with a half-life of 1.41×10^{10} years; and potassium 40 with an half-life of 1.28×10^{9} years. According to Tufail *et al*. (2006), phosphate and potassium-based inorganic fertilizers are prepared from rocks and the use of these is known to contribute to radioactivity in agricultural crops (which contain ^{238}U and ^{40}K, respectively).

Cosmogenic-origin radionuclides have been detected mainly in organic materials or substrata. Some of the examples include: carbon-14 with a half-life of 5730 years; tritium or hydrogen-3 with a half-life of 12.3 years; and beryllium with a half-life of just 53 days.

Finally, the third important type of radionuclides is those produced by human interventions. These are introduced into nature by fission reactions or via nuclear reactors, nuclear weapons
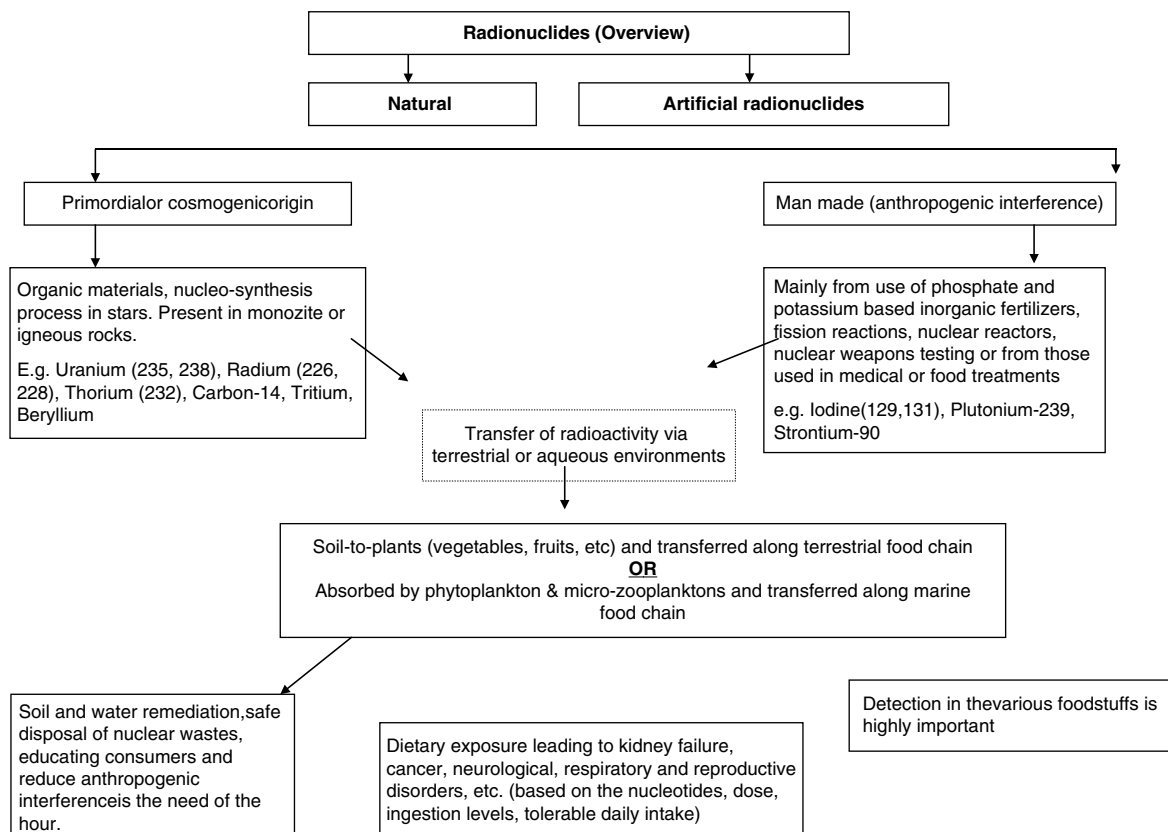
Figure 15.1 An overview of the presence of natural and artificial radionuclides in the atmosphere.

testing or from those used in medical or food treatments. Some examples include: iodine-131 with a half-life of nearly 8 days; iodine-129 with a half-life of 1.57×10^7 years; caesium-137 with a half-life of nearly 30 years; plutonium-239 with a half-life of nearly 2.41×10^4 years; and strontium-90 with a half-life of nearly 29 years.

Generally, radionuclides are symbolized based on the element and their atomic weight. For example: 210-Po or ^{210}Po for polonium, U-235 or ^{235}U and U-238 or ^{238}U for uranium, Th 232 or ^{232}Th for thorium, ^{226}Ra or radium 226 for radium, potassium 40 or ^{40}K for radioactive potassium, caesium 137 or ^{137}Cs, H-3 or ^{3}H for radioactive hydrogen, etc. Although there are several natural and human introduce ionizing radiations present in the environment, alpha-emitting radioisotopes

are of high concern, especially regarding food. Some γ-ray emitting radionuclides include uranium and thorium series, ^{137}Cs and ^{40}K. The presence of natural radionuclides is highly specific to selected regions and the concentration levels can vary between different geographical locations. Commercially, several radionuclides are available and used in medicine, industries and in food preservation. Some of these include ^{60}Co, ^{137}Cs, ^{133}Ba, ^{109}Cd and ^{22}Na.

Several studies have reported on the varied levels of radionuclide concentration including those of uranium and thorium series, ^{226}Ra and ^{90}Sr in the environment (Osborne, 1963; Beasely and Palmer, 1966; Schell *et al.*, 1973; IAEA, 2003; Steinhauser *et al.*, 2013). Reports are available on the uniform distribution of these nuclides in soil

(Ramzaev *et al*., 2006; Belivermiş *et al*., 2010). However, studies on vertical distribution are important as mining and fossil fuel combustion are capable of leading to high natural radionuclide levels in soil. Recently, Belivermiş (2012) reported on the vertical distribution of radionuclides (in Istanbul, Turkey environment) such as ^{137}Cs, ^{40}K, ^{232}Th and ^{226}Ra in soil of 30 cm depth. Even more than 20 years after the Chernobyl disaster, ^{137}Cs still remained (55%) in the top 10 cm of soil.

15.3   Historical background of radioactivity

The discovery of radioactivity can be dated to as early as the 18th century (1896) when Henri Becquerel investigated uranium salts for phosphorescence. During this era, many eminent researchers emerged, including Pierre Curie, Marie Curie, Ernest Rutherford, Jöns Jacob Berzelius, Adair Crawford, William Cruikshank, Sir Humphry Davy, Demarcay, Debierne, and Hans Geiger, to name a few. Words such as 'radiation', 'radionuclides' and 'radiation exposure' are terms which have been constantly used since the discovery of radioactive elements. Apart from the commonly highlighted harmful effects, several advantages are also known which have been useful to mankind. The application of gamma rays, electron beams, ultraviolet and X-rays (high and low energy) have been used for food preservation, and some of the radioisotopes are used in nuclear medicine, genetic studies, mineral component detection for mining, detection of environmental pollutants and power generation.

However, harmful effects occur when these radionuclides enter the environment or get into the food chain. Although more than a dozen nuclear disaster incidences (rather accidents) have been reported worldwide, there are several unforgettable incidences. In fact, the most devastating incidence mankind will ever remember is that of Hiroshima (use of uranium) and Nagasaki (use of plutonium) when atomic bombs were used for the first time (during World War II in 1945). Some of the other incidents include: Sellafield accident in United Kingdom (1957); Three Mile Island accident in United States (1979); Chernobyl disaster in Ukraine (1986); radiotherapy accident in Costa Rica (1996); radiation exposure in Samut Prakan, Thailand (2000); and the Mayapuri incidence in India (2010). Among these accidents, the most recent disaster witnessed by mankind is that of Fukushima Daiichi nuclear accident in Japan (2011), wherein nearly 18 PBq of ^{134}Cs and 15 PBq of ^{137}Cs and 160 PBq of ^{131}I fallout in the surrounding environment was detected (Hamada and Ogino, 2012; Hamada *et al*., 2012). At least some of these accidents occurred as a direct result of human negligence or human interventions, allowing radionuclides to enter the environment easily and affect living entities.

15.3.1   Most recent large-scale radiation release

The two most devastating large-scale radiation releases of the recent past witnessed by mankind occurred in Chernobyl (on 26 April 1986) and in Fukushima (on 11 March 2001).

The nuclear reactor accident at Chernobyl occurred in Reactor 4 of the Chernobyl nuclear power plant, where radionuclide release occurred mainly during the initial 10-day period of time. The most important radionuclides in terms of radiation exposure to the general population included ^{131}I, ^{134}Cs and ^{137}Cs. According to UNSCEAR (2008) estimates, the release of 1760 PBq, 47 PBq and 85 PBq occurred for these radionuclides, respectively. The plant was located in Ukraine (part of the Soviet Union during the accident), and this country together with the Russian Federation and Belarus were the most affected (Cardis *et al*., 2005). In addition, most of the countries in the Northern Hemisphere were also affected by the radioactive cloud due to the prolonged release of radionuclides coupled with changing meteorological conditions, mainly due to rainfall (UNSCEAR, 1988). The radiation was fatal and led to radiation injuries for the local

populations within the short span of weeks. The estimation of the consequences of the Chernobyl accident on human health generated much debate with confronting points of view, as can be seen from the discussions raised by BELRAD (2012) and Jargin (2010).

Children are most susceptible as they drink proportionally more milk than adults and they have a smaller thyroid mass, which is the part of the body where iodine concentrates (UNSCEAR, 1988). The main pathway of human exposure to ^{131}I was via drinking milk from cows fed with contaminated pasture grass. An epidemiological study performed among the population of affected areas in Belarus and the Russian Federation after 12 years demonstrated a strong relationship between radiation dose levels to the thyroid in childhood with enhanced thyroid cancer risk (Cardis *et al.*, 2005). As for radio-caesium, the most significant sources of internal exposure were milk and meat (UNSCEAR, 1988).

The second major recent incident is that of the Fukushima Daiichi nuclear power facility, located in the Fukushima Prefecture in Japan, which suffered major structural damage from a magnitude of 9.0 earthquake followed by a tsunami, which led to significant release of radioactivity to the environment (Dauer *et al.*, 2001). High amounts of radioactivity were released in the surrounding atmosphere as well as to the Pacific Ocean. Most of the radioactivity was transported directly to the sea, as seawater is used to cool the reactor. Later a continued input of ^{137}Cs into the sea was recorded due to leaching from contaminated surface land and by liberation of radio-caesium particles from soil sediments (IRSN, 2011). Since wind blows from the west in Japan, most of the aerial deposition was directed towards the sea; however, the radioactive cloud was also observed to be directed towards northwest and southwest regions of the Fukushima Daiichi site (Wakeford, 2011). However, the radiation dose exposure by the general population was considered to be about one-tenth that of the Chernobyl accident (Tamaki and Shishido, 2011). The isotopes ^{131}I, [134]Cs and ^{137}Cs were the main radioactive products released to the environment (IRSN, 2011).

Nearly 7 lakhs and 70,000 Tera-Becquerel (Bq) of radioactive materials were reportedly released in the environment following the Fukushima Daiichi nuclear accident. These radionuclides was reported to lead to low-dose radiation exposure (internal or external) among 2 million Fukushima residents (Tomonaga, 2012). Since this incident, several research studies have been undertaken in and around the Fukushima reactor accident sites relevant to the distribution of these harmful radionuclides in the environment (air, water and soil, including those of marine biota) (Masson *et al.*, 2011; Norman *et al.*, 2011; Hamada and Ogino, 2012; Higaki *et al.*, 2012; Hirose, 2012; Kato *et al.*, 2012; Madigan *et al.*, 2012; Shimura *et al.*, 2012; Steinhauser *et al.*, 2013).

From the food safety point of view, contamination of sea or ocean water can have an effect on seafood. Effects along the food chain can be expected to increase with time (especially in predator fishes) due to accumulation of radioactivity through the trophic chain (IRSN, 2011). On August 2011, 15 Pacific Blue fin tunas caught in California demonstrated a presence of [134]Cs ($4 \, Bq \, kg^{-1}$) and [137]Cs ($6.3 \, Bq \, kg^{-1}$). This species migrates from Japan to California. Even though these radio-caesium concentrations were not considered dangerous for human consumption, these results highlight how far radionuclides can be transported by migratory animals used for human consumption (Madigan *et al.*, 2012). In another report, the contamination affected tap water in Tokyo (230 km from the accident site), where concentrations of [131]I ($210 \, Bq \, kg^{-1}$ on March 22) were detected (Unno *et al.*, 2012). In addition, a preliminary assessment conducted in the Fukushima Prefecture (during July) on levels of radio-caesium contamination of whole-day meals, vegetables from local vendors and tap water concluded that dose levels were lower than the permissible dose (Koizumi *et al.*, 2012).

As in Chernobyl, the accumulation of ^{131}I in the thyroid gland was prevented by distribution of stable iodine tablets (or syrup for children). A survey of children from badly affected areas regarding the presence of ^{131}I did not reveal

thyroid dose rates of concern (Wakeford, 2011). As the majority of the Japanese population have a natural intake of iodine in their diet, the Japanese Society of Nuclear Medicine announced later that thyroid block was not required for children (Tamaki and Shishido, 2011). Further, contamination of breast milk with ^{131}I was also detected in 7 out of 23 women (30%) during April, which was undetectable by May 15. However, it was opined that nursing infants might also have been exposed to larger doses, the possible route of contamination being contaminated tap water and vegetables (Unno *et al.*, 2012). In addition, Koizumi *et al.* (2012) reported that shiitake mushrooms (*Lentinula edodes*) were the only food item in the market of Fukushima Prefecture which had radioactivity levels higher than those recommended.

15.4 Radionuclides and the food chain

It is imperative to monitor the levels at which a radionuclide is present in a foodstuff which is used for human consumption or as feed for livestock. Providing adequate information relevant to health risks associated with exposure to radionuclides via dietary intake is very important for the general population. According to WHO recommendations (1985), assessing the intake of food contaminants or other dietary elements should combine information on specific contaminants along with providing information on the individual or household consumption records, which should consider total diet studies, duplicate diet studies and diary studies.

Above all, the general population can either be exposed externally to a radiation source or internally by a radioactive material which has entered the body by absorption through skin wounds, inhalation or consumption of contaminated food and water. All humans receive a small dose of radiation from the surrounding environment, referred to as background radiation, with a typical dose estimated as 2.4 mSv per year (including 0.29 mSv per year due to ingestion),

with typical dose rates of 0.3–1.5 μSv h^{-1} (UNSCEAR, 2011).

Total radiation exposure in humans is mainly from food chain transfer. Most of the ingested radionuclides (via dietary habits or inhalation) builds up in the bones or other vital tissues inside the body. In fact, some of the radionuclides such as ^{137}Cs and ^{90}Sr can enter the geo-chemical cycle or food chain via organic debris of the dead plants, especially in agricultural areas (Gastberger *et al.*, 2001; Lettner *et al.*, 2007). Generally, humans become exposed to radiation from natural radionuclides (uranium and thorium series) due to their capacity to dissolve in water, enter water bodies and hence contaminate food stuffs (raw materials) (Chen *et al.*, 2005).

Research work accomplished on the actual mechanism involved in accumulation of radionuclides and transfer to plants and human population is scarce (Mitchell, 1974; Gaso *et al.*, 2000). Generally, two mechanisms can be involved when radionuclide contamination occur in a plant or vegetation. This can be either via root uptake or by direct aerial deposition of radionuclide fallout on the plants; the accumulation and transfer of radionuclides in sea or aquatic biomes can differ.

Transfer of radioactivity to a plant part or tissue is referred to as transfer factor (TF). Providing details on the soil-to-plant transfer factor (TF) is important for environmental safety assessment required in and around nuclear facilities (IAEA, 1994). Several studies have been undertaken on various foodstuffs (e.g. vegetables cereals, tubers, herbs, etc.; Table 15.1) relevant to the transfer factor of naturally occurring radionuclides (Brown and Simmonds, 1995; Ham *et al.*, 2001; Ramaswami *et al.*, 2001; Ewers *et al.*, 2003; Sheppard *et al.*, 2006; IAEA, 2010; Antovic and Antovic, 2011; Karunakara *et al.*, 2013). In general, transfer factor (TF) is expressed as:

$$TF = \frac{\text{Activity concentration in plant}}{\text{Activity concentration of substrate}}$$

where both quantities are measured in units of Bq kg^{-1} dry weight.

Table 15.1 Recent reports highlighting the presence of radionuclides in various foodstuffs.

Radionuclides	Food products evaluated	Reference
^{210}Po and ^{210}Pb	Different fish species, seafood, molluscs, crustaceans, in oysters, cereals, mussels, legumes	Bhat et al. (2005); Carvalho (2011); Carvalho et al. (2011); Turtiainen et al. (2011); Çatal et al. (2012); Musthafa and Krishnamoorthy (2012); Uddin et al. (2012); Khan and Wesley (2013); Lee and Wang (2013)
^{210}Po, ^{210}Pb, ^{40}K and ^{137}Cs	Edible wild berries and mushrooms	Gwynn et al. (2013)
^{134}Cs and ^{137}Cs	Coconut, pandanus, breadfruit, in aquatic food webs, milk, sugar and confectioneries (3rd food group), vegetables, seaweeds and mushrooms, fish, shellfish and processed seafood, in fish and molluscs, rice and in paddy field	Changizi et al. (2012); Harada and Masanori (2012); Joshi et al. (2012); Endo et al. (2013); Merz et al. (2013); Miyazaki et al. (2013); Peters et al. (2013); Rowan (2013)
^{137}Cs, ^{40}K, ^{226}Ra, ^{232}Th	Wheat, millet, lentils, potato, cauliflower, rice	Khan et al. (2010); Okuda et al. (2013)
^{137}Cs, ^{90}Sr, ^{40}K	Grains and grain products	Solecki and Kruk (2011)
^{226}Ra, ^{228}Ra, ^{210}Pb, ^{40}K and ^{137}Cs	Rice	Karunakara et al. (2013)
^{238}U, ^{232}Th, ^{222}Rn, and ^{220}Rn	Various locally produced olive oil samples	Misdaq and Touti (2012)
^{40}K, ^{232}Th and ^{226}Ra	In powdered infant's milk	Al-Zahrani (2012)
^{40}K, ^{226}Ra and ^{232}Th	Wheat	Tufail et al. (2010)
^{226}Ra, ^{232}Th, and ^{40}K	Breakfast cereals	Alrefae et al. (2012)
^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs	Commercial fish	Korkmaz et al. (2012)
^{238}U, ^{226}Ra, ^{228}Ra, ^{232}Th, and ^{40}K	Shellfish (molluscs and crustacean-based food)	Khandaker et al. (2013)
^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs	Vegetables and fruits, marine fish	Korkmaz et al. (2012); Canbazolu and Douru (2013)
^{232}Th, ^{238}U, ^{40}K and ^{137}Cs	Tea, cabbage, orange, kiwi	Keser et al. (2011)
^{134}Cs, ^{137}Cs, ^{131}I	Various foods	Hayashi and Midorikawa (2011)
^{137}Cs and ^{226}Ra	Fish species, rice	Antovic and Antovic (2001); Uchida et al. (2007)
^{137}Cs and ^{40}K	Dried pulses (33), fresh pulses (15), leafy vegetables (52), fruit vegetables (7), flower vegetables (44), root crops (18), tubers (16), ferns (93), mushrooms, vegetables, grass and milk	Panchal et al. (2011); Tagami and Uchida (2013)
^{40}K, ^{232}Th and ^{226}Ra	Powdered infant's milk	Al-Zahrani (2012)
^{238}U, ^{234}U, ^{230}Th, ^{226}Ra, ^{210}Po, ^{232}Th, ^{238}Th, ^{210}Pb and ^{228}Ra	Tea	Görür et al. (2012)
^{238}U, ^{234}U, ^{230}Th, ^{226}Ra, ^{210}Pb and ^{210}Po	Hay, silage and milk	Strok and Smodis (2012)
^{90}Str	Soil and vegetation samples	Steinhauser (2013)
Uranium	Spinach, drinking water, cereal grains, zebra fish;	Augustine et al. (2012); Neves et al. (2012); Gramss and Voigt (2013); Kishi et al. (2013); Larivière et al. (2013)
^{228}Ra	Fruits, leafy vegetables, tubers and roots, palm embryo, cereals,	Ewers et al. (2003); Sheppard et al. (2006); Ross et al. (2013)
Thorium	Food, drinking water, vegetables	da Costa Lauria et al. (2012); Sathyapria et al. (2012)
Uranium, thorium, and decay products	Medicinal plants, chicken, meat and egg	Oufni et al. (2011); Jeambrun et al. (2012); Jha et al. (2012)

There is a high chance that foods encompassing high background radiation can effortlessly enter the market chain (food or agriculture) where radionuclides might not have been deposited previously. To evaluate radionuclide concentrations and estimate their deposition through terrestrial food chains, a dynamic food chain model was developed by Brown and Simmonds (1995).

With regard to safety, assessing the influence in an ecosystem includes concentration of radionuclides absorbed by plants from soil (Blanco Rodriguez et al., 2002; Pulhani et al., 2005). Soil tends to play a major role compared to water bodies. The occurrence of soil-derived natural radionuclides entering the food chain depends on soil characteristics and properties such as clay content, pH, minerals, fertilizers and organic matter contents (Jibiri et al., 2007a, b; Hegazy and Emam, 2011; Soliman et al., 2011). The presence of radionuclides in upper layers of soil presents serious hazards to the surrounding environment and human health, as there are elevated chances of their integration into the human food chain. Al-Masri et al. (2008) reported augmentation in the radionuclide concentrations in plant tissues directly corresponding to their increased concentration in soil. According to Abrahams (2002), the uptake of radionuclides by plants vary among a range of plant species, and can therefore affect the detected concentrations in food products.

This opinion also holds true in the case of marine ecosystem. In certain instances, plants and animals have been recommended to be used as bio- indicators to examine the atmospheric fallout of radionuclides (Skwarzec and Jakusik, 2003; Somashekarappa et al., 1996; Karunakara et al., 2000; Vega et al., 2012). The influence of food processing methods such as cooking on radionuclide contents has been excellently reviewed by Green and Wilkins (1996), highlighting the gaps where future information is necessary.

According to Karunakara et al. (2013), critical pathways of radionuclides in foods can differ between Asian countries and Western countries, which are attributable to differences in diets and agricultural products. Of late, there are many studies being conducted and reported on the presence of radionuclides in food. The most-studied food sample of plant origin is that of mushrooms. There are several natural and artificial radionuclides (e.g. ^{40}K, ^{137}Cs) detected in mushrooms collected worldwide (Baeza et al., 2005; Mietelski et al., 2010). Reports are also available describing high levels of artificial radionuclides observed in mushrooms at sites of nuclear weapons testing and nuclear power plants (Taira et al., 2011). Further, radionuclides in edible wild-grown and cultivated mushrooms have been reviewed recently (Falandysz and Borovička, 2013). In this review, authors have stated that mushrooms can absorb ^{137}Cs from soil surface, deeper soil layers and decaying litter which is related to fungal biology and placement of mycelium.

Hamada and Omigo (2012) reported on the presence of various levels of radio-caesium and radio-iodine in different foodstuffs such as milk, meat and cereals and those of water. As an example of this study, exceeded limits of planar radionuclide ventriculography (PRV) for radio-caesium was observed in brown rice compared to polished rice. The observed enhanced limits were irrespective of the three-stage strategies of cultivation restrictions, pre- and post-harvest surveys. Contamination in foods (total 445 samples) by determining the radio-caesium activity ratio (^{134}Cs/^{137}Cs) has been reported by Merz et al. (2013). Accordingly, these authors attempted to analyse the abundance of radionuclides in food as well as their radioactive signature and potential contributors to their source. Their results showed an average of activity ratio of 0.98 (^{134}Cs/^{137}Cs) in all samples.

Since the Fukushima Daiichi nuclear power plant incidence in March 2011, many active research works have been undertaken with regard to detection of radioactive contaminants in food. Detection of radioactive contamination (via ^{131}I, ^{134}Cs and ^{137}Cs fission products) in foodstuffs (total 442 samples) imported from Japan (to Thailand) after the Fukushima accident was

reported by Itthipoonthanakorn *et al.* (2013). Their results revealed the presence of high levels of ^{131}I (half-life of 8 days) initially, which was attributed to its gaseous nature and easy dispersal from the nuclear accident compared to the solid ^{134}Cs and ^{137}Cs. The observed activity in various foodstuffs for ^{131}I, ^{134}Cs and ^{137}Cs was within the range 0.63–15.25, 1.45–44.70 and 0.45–51.10 Bq kg^{-1} fresh weight, respectively. The authors concluded by stating that the observed concentration levels are within the safe limits of Thailand standard for contaminated foods. In addition, Kishi *et al.* (2013) have reported on the uranium contents in 9 spinach samples, the level of which was <10 μg kg^{-1} (samples bought during April and May 2011 during Fukushima accident). The authors concluded that U levels were low compared to the provisional regulatory limit in Japan.

These are just a few examples highlighting the ongoing interest among researchers to provide basic informations on the contaminant levels of radionuclides in food.

15.5   Measurement of radionuclides in food

Radioactivity is generally measured and represented in the units Becquerel (Bq), which is equal to 1 decay per second; in other words, 1 Bq is the activity of a known amount of radioactive material in which one nucleus decays per second. The units of kiloBecquerel or kBq (10^3 Bq), megaBecquerel or MBq (10^6 Bq), gigaGecquerel or GBq (10^9 Bq) and teraBecquerel or TBq (10^{12} Bq) are also unsed. The Curie unit was used prior to the use of Bq, where a Curie or 1 Ci is equal to 37 GBq (1 Ci = 3.7×10^{10} s^{-1}) (Allisy, 1995). For ionizing radiations, the unit used is Sievert (Sv) or Gray (Gy) for absorbed dose and equivalent doses, respectively (1 Gy = 100 rad and 1 Sv = 100 rem, where rem is a unit used to measure biological damage from radiation). Using radiation counting systems, activity is measured in units of dpm (disintegrations per minute) or as cpm (counts per minute). Generally radiation is

measured by various instruments such as: liquid scintillation counter (LSC); standard proportional counter; multichannel analyser system equipped with germanium detector, a silicon-type detector, a sodium iodide crystal and photomultiplier tube or a Geiger Counter equipped with a tube or probe; or by neutron REM meter with proportional counter and radon detectors.

Meanwhile, the effective dose from radionuclides in a food commodity is calculated by various equations and methods. This may change based on the methods adopted or on the samples studied. Some common examples are described in the following.

The most commonly used (Samavat *et al.*, 2006; Jibiri *et al.*, 2007 b) is:

$$D = D_{\mathrm{f}} \times U \times C_{\mathrm{d}} \times h$$

where D is effective dose (μSv year^{-1}); D_{f} is dose coefficient (μSv Bq^{-1}); U is amount of food (kg) consumed annually; C_{d} is radionuclide content of dried food; and h is the ratio of dried to fresh foods. The quantity $C_{\mathrm{d}} \times h$ is the radionuclide content in fresh food (expressed as Bq kg^{-1}).

In fish and other seafoods, the following calculation is used (IAEA 1995) instead:

$$D_{\mathrm{Po}} = C_{\mathrm{b}} \times F_{\mathrm{c}} \times F_{\mathrm{h}} \times F_{\mathrm{e}} \times D_{\mathrm{f}} \times 4.3 \times 10^{-7}$$

where D_{Po} is the effective dose of ^{210}Po; C_{b} is ^{210}Po activity (Bq kg^{-1} ww); F_{c} is catch of fish (kg year^{-1}); F_{h} is catch used for human consumption; F_{e} is catch fraction that is eaten; D_{f} is the delay factor relevant to catch and consumption time; and 4.3×10^{-7} is the factor used for effective dose calculations (ingestion dose coefficient).

In the case of oysters, the internal dose of ingestion of ^{210}Po is calculated based on the following equation (International Commission on Radiological Protection, 1996; Lee and Wang, 2013):

$$D = K \times G \times C$$

where D is effective dose via ingestion (mSv); K is ingesting dose conversion factor of ^{210}Po

radionuclide ($1.2 \times 10^3\,\text{mSv}\,\text{Bq}^{-1}$); G is oyster consumption per year; and C is the summation of the weighted average activity concentration of ^{210}Po radionuclide ($25.9\,\text{Bq}\,\text{kg}^{-1}$).

Radioactive ^{210}Po and ^{210}Pb in seafood or vegetation samples can be analysed by acid leaching (Jia *et al.*, 2001; Bhat *et al.*, 2005). ^{210}Po or ^{210}Pb concentrations can be determined using the standard silver disc technique or the electrochemical deposition method (Flynn, 1968; Iyengar *et al.*, 1990; Bhat *et al.*, 2005). Generally, acid-digested samples are deposited on a brightly polished silver disc by employing electro-chemical exchange method, and counted on both sides for ^{210}Po activity using a ZnS (Ag) alpha counter. From the counts, the activity A is calculated as per the equation:

$$A = \left[S \pm SD\right]\frac{100}{E} \times \frac{100}{E_\text{p}} \times \frac{100}{W} \times \frac{100 - M}{100}$$

where A is activity ($\text{Bq}\,\text{kg}^{-1}$); S is net counts per second; SD is standard deviation; E is efficiency (%) of the alpha counter; E_p is plating efficiency (%); W is the weight of the dry sample; and M is the moisture content (%) of the sample.

Daily activity intake of ^{210}Po and ^{210}Pb have also been calculated using (Khan and Wesley, 2011):

$$AI = CR \times IR \times MF \times FI$$

where AI is activity intake in Bq; CR is radionuclide concentration in edible tissue ($\text{Bq}\,\text{kg}^{-1}$); IR is ingestion rate ($\text{kg}\,\text{day}^{-1}$); MF is the modifying factor due to decay of ^{210}Po between catch and consumption; and FI is the real fraction consumed.

Radioactivity in milk, meat and offal samples has been calculated for technetium-99, ruthenium-106, cerium-144, promethium-147 and plutonium-241 using the following equation (RIFE, 2010):

$$C_\text{m} = F_\text{m}C_\text{a}Q_\text{f} \quad \text{and} \quad C_\text{f} = F_\text{f}C_\text{a}Q_\text{f}$$

where C_m is concentration in milk ($\text{Bq}\,\text{L}^{-1}$); C_f is concentration in meat or offal ($\text{Bq}\,\text{kg}^{-1}$ fresh);

F_m is the fraction of the animal's daily intake by ingestion transferred to milk ($\text{day}\,\text{L}^{-1}$); F_f is the fraction of the animal's daily intake by ingestion transferred to meat or offal ($\text{day}\,\text{kg}^{-1}$ fresh); C_a is the concentration in fodder ($\text{Bq}\,\text{kg}^{-1}$ dry); and Q_f is the amount of fodder eaten per day (kg dry day^{-1}).

Neves *et al.* (2012) provided information on calculating hazard quotient (HQ, dimensionless) in adults prone to uranium exposure from foodstuffs ingestion, based on USEPA (1997, 2000) and Integrated Risk Information Systems (2008):

$$HQ = E_\text{d,ing} = RD$$

where RD is oral reference dose from the Integrated Risk Information System (2008) database ($0.003\,\text{mg}\,\text{kg}^{-1}$ day) and $E_\text{d,ing}$ is estimated exposure dose from ingestion pathway ($\text{mg}\,\text{kg}^{-1}$ day), defined:

$$E_\text{d,ing} = \frac{C \times IR \times E_\text{d} \times E_\text{f}}{B_\text{w}} \times T_\text{e}$$

where C is element concentration in the foodstuff ($\text{mg}\,\text{kg}^{-1}$); IR is food ingestion rate (kg/person/day); E_d is exposure duration (50 years); E_f is exposure frequency ($\text{day}\,\text{year}^{-1}$); B_w is adult body weight (c. 70 kg); and T_e is average exposure time for non-cancer risks (which is 50×365 days).

To estimate the radiation dose delivered to the general public, external hazard index H_ex and internal hazard index H_in are calculated. The external and internal hazard index is calculated for ^{226}Ra, ^{232}Th and ^{40}K using the following equations (NEA-OECD, 1979; Krieger, 1981; Beretka and Mathew, 1985):

$$H_\text{ex} = \frac{C_\text{Ra}}{370} + \frac{C_\text{Th}}{259} + \frac{C_\text{K}}{4810} \quad \text{and}$$

$$H_\text{in} = \frac{C_\text{Ra}}{185} + \frac{C_\text{Th}}{259} + \frac{C_\text{K}}{4810}$$

where C_Ra, C_Th and C_K are activity concentrations of ^{226}Ra, ^{232}Th and ^{40}K, respectively.

In addition, the approach of concentration ratio CR has been used to predict radionuclides

concentration in natural biota (terrestrial and aquatic organisms). The *CR* method is based on the hypothesis that the activity of radionuclides *R* is in bio-geochemical equilibrium with the activity concentration in the environment. Two approaches defining *CR* have been detailed by Wood *et al.* (2010). The first is relevant to aquatic ecosystems (Hosseini *et al.*, 2008) and the second is based on fresh mass (fm) of an organism to that of soil dry mass (dm) (Beresford *et al.*, 2008). For aquatic ecosystems, CR is defined:

$$CR = \frac{\text{Activity concentration of } R \text{ in biota whole body } \left(\text{Bq kg}^{-1} \text{ fm} \right)}{\text{Activity concentration of } R \text{ in filtered water } \left(\text{Bq L}^{-1} \right)}$$

and for terrestrial ecosystems, CR is defined:

$$CR = \frac{\text{Activity concentration of } R \text{ in biota whole body } \left(\text{Bq kg}^{-1} \text{ fm} \right)}{\text{Activity concentration of } R \text{ in soil } \left(\text{Bq kg}^{-1} \text{ dm} \right)}.$$

Based on the WHO and FAO recommendations on guideline levels to the Codex Alimentarius Commission, the following is used to calculate radioactivity levels with a reference level of 5 mSv dose:

$$\text{Level} = \frac{\text{RLD}}{m \times d}$$

where RLD is reference level of dose (Sv); *m* is mass of food consumed (kg); and *d* is dose per unit intake factor (Sv Bq^{-1}).

Radionuclides such as ^{226}Ra, ^{232}Th and ^{40}K are generally not uniformly distrusted in soil and their concentration varies. To identify and calculate the actual activity concentrations, radium equivalent activity (R_{aeq}) calculation is used (Beretka and Mathew, 1995). According to the Organization for Economic Cooperation and Development (OECD, 1979), maximum R_{aeq} in a sample should be <370 Bq kg^{-1}. R_{aeq} is calculated as:

$$R_{\text{aeq}} = A_{\text{Ra}} + \frac{10}{7} A_{\text{Th}} + \frac{10}{130} A_{\text{K}}$$

where A_{Ra}, A_{Th} and A_{K} are the activity concentrations of ^{226}Ra, ^{232}Th and ^{40}K, respectively.

In addition to these, uranium (^{238}U) and thorium (^{232}Th) can also be determined in acid-digested foodstuffs by using inductively coupled plasma mass spectrometry (ICP-MS) or inductively coupled plasma atomic emission spectrometry (ICP-AES) and by instrumental neutron activation analysis. Various methods have been adopted for determination of uranium, radium and thorium in food and drinking water (da Costa Lauria *et al.*, 2012). As per ICRP (1996), the recommended dose conversion coefficient for ^{226}Ra, ^{228}Ra, ^{228}Th and ^{40}K is 0.23, 0.8, 0.072 and 0.0062 μSv Bq^{-1}, respectively.

European Union comments and proposed draft revision of guideline levels for radionuclides in foods (EU Comments, Moscow, 2013) clearly state that 'The EU agrees with the approach to exclude radionuclides of natural origin from the setting of guideline levels. However the EU is of the opinion that it is appropriate to continue monitoring the presence of radionuclides of natural origin and to assess the potential risk for public health.' In addition, as an outcome of the above meeting, it was agreed on the principles developed by International Commission on Radiological Protection (ICRP Publication 111) on the 'Management of contaminated foodstuffs and other commodities' to be a basis for the revision of the current 'Fact Sheet of the Codex Secretariat of May 2011'.

Codex (2011) has given guidelines for presence of radionuclides (^{90}Sr, ^{131}I, ^{137}Cs, ^{134}Cs, ^{239}PU and ^{241}Am) in food and feed following a nuclear emergency. These guidelines are intended to be used in international trade. Bullard (2012) provided details on various emergency responses, medical management of victims exposed to radiological elements and planning and preparedness that need to be considered in case of a nuclear disaster or exposure to radioactive elements. Details on the safety regulations, policy

for execution of monitoring surveys and restrictions of food and water implemented post-Fukushima nuclear accident have been summarized very effectively by Hamada *et al.* (2012), which may be very useful following any future nuclear emergency as well as forming a basis for discussion of the optimization of future radiation protection strategies worldwide.

HPGe and NaI (Tl) scintillation detectors are usually employed for food inspection after nuclear accidents. Due to their limitations (long time required for detection of the concentrations as well as non-ability to detect radionuclides such as ^{90}Sr) however, Sato *et al.* (2013) designed a novel detector based on Monte Carlo simulations for measuring radioactivity which can count rates using 10 layers of proportional counters (separated by walls that absorb beta particles). According to the inventors, this detector was based on the existing proportional counter at AIST (Sato *et al.*, 2012). Results obtained for simulation demonstrated that the counter detected activity of ^{90}Sr in food samples without using any chemical processes. The authors have concluded that this counter would of immense use in the detection of radionuclides such as ^{137}Cs, ^{90}Sr, ^{40}K and ^{90}Y.

In Sections 15.6–15.8 we describe some of the important radionuclides encountered and their health effects and concentration levels in food.

15.6 ^{210}Po and ^{210}Pb (polonium and lead) in food

^{210}Po and ^{210}Pb are the most important naturally occurring radionuclides found abundantly in nature (sea and soil). ^{210}Po is a high-energy alpha emitter with a half-life of 138 days, while ^{210}Pb is a beta emitter with a half-life of 22.2 years (UNSCEAR, 1993). 210-polonium is considered the most toxic radionuclides to humans (McDonald *et al.*, 1986) with a maximum ingestible permissible limit being 1.1×10^3 Bq (Weast and Astle, 1982). According to UNSCEAR (2000), the annual intake of ^{210}Po in the diet should not exceed 58 Bq.

^{210}Po and ^{210}Pb are the daughter (decay) products of ^{238}U. The hazard nature of ^{210}Po is comparable to plutonium, and it is nearly five times more toxic than ^{226}Ra (McDonald *et al.*, 1986). Available reports indicate discharge from petroleum (oil and gas) industries, combustion of fuel and waste discharge post-phosphate-rock processing to be the main contributors of accumulating ^{210}Po and ^{210}Pb in the environment, especially in marine biomes and estuaries (Carvalho and Fowler, 1993; McDonald *et al.*, 1996; Godoy *et al.*, 2008; Villa *et al.*, 2009; Carvalho *et al.*, 2010). Additionally, ^{222}Rn released from the Earth's crust to the surrounding environment is also considered as a major source of ^{210}Po and ^{210}Pb (Skwarzec and Bojanowski, 1988; Yamamoto *et al.*, 1994; Dahlgaard, 1996). Furthermore, tetraethyl lead in petrol, burning of fossil fuels and the use of superphosphate fertilizers can contribute to ^{210}Pb and ^{210}Po (Santos *et al.*, 1989; Amaral *et al.*, 1992). In addition, Narayana and Rajashekara (2010) have opined that ^{210}Po tends to act as a nutrient element and hence its concentration can deplete rapidly in soil compared to ^{210}Pb.

In areas of normal background radiation, the effective dose equivalent of ^{210}Po is reported to be approximately 130 μSv (UNSCEAR, 1982). According to Cherry and Heyraud (1981), in marine organisms ^{210}Po has higher affinity for organic matter than ^{210}Pb. There is much information available where the presence of ^{210}Po has been reported in vegetation and plant produce used for human consumption. The presence of ^{210}Po in orchids, moss, mushrooms, vegetables, seaweeds, cereals and grains from different geographical locations have been reported (Somashekarappa *et al.*, 1996; Bukhari *et al.*, 2003, Skwarzec and Jacusik, 2003).

Bioaccumulation of ^{210}Po in different parts of coastal sand dune legumes (*Canavalia cathartica* and *C. maritima*) has been reported by Bhat *et al.* (2005). Accordingly, rhizosphere sand samples demonstrated high ^{210}Po activity (5.78–5.88 Bq kg^{-1}) while mature beans had the lowest activity (0.13–0.20 Bq kg^{-1}), indicating their presence. Authors have suggested that these coastal legumes, widely distributed in the

pan-tropical regions of the world, could be effective bio-indicators to identify radionuclide accumulation in the coastal biomes. ^{210}Po has also been detected in drinking as well as in mineral water and in beer (Skwarzec *et al.*, 2004; Forte *et al.*, 2007; Vesterbacka, 2007). Various views have been put forward on bioaccumulation of ^{210}Po and ^{210}Pb in food. According to Parfenov (1974), radionuclides such as ^{210}Po can be transferred directly by root absorption from soil. In contrast, Simon and Ibrahim (1987) have stated ^{210}Po is excluded from root absorption from soil. On the other hand, aerial deposition and absorption by leaves has been proposed by Santos *et al.* (1990). Apart from vegetation, ^{210}Po is known to accrue in high amounts in marine organisms (flora and fauna) and is accountable for internal radiation dose. Also, high ^{210}Po concentration (along with uranium-series radionuclides) has been reported in kidney and liver of buffalo, magpie geese and swine (Davy and Conway, 1974; Martin *et al.*, 1998).

The high radiation dose accumulated by marine organisms is attributed to the presence of naturally occurring uranium series compounds such as that of ^{210}Po (Cherry *et al.*, 1994; Stepnowski and Skwarzec, 2000). As ^{210}Po is not readily soluble in seawater, plankton can easily accumulate radionuclides and pass them on to marine organisms which feed on them and later to humans (Tanaka and Tsunogai, 1983; Carvalho and Fowler, 1994; Suriyanarayanan *et al.*, 2008; Strok and Smodis, 2011). In fact, this is the reason that people consuming high level of seafoods can accumulate ^{210}Po at higher than average levels (Heyraud and Cherry, 1979; Cherry and Heyraud, 1981; Carvalho, 1988, 1995; UNSCEAR, 1993; Aarkrog *et al.*, 1997; Bellamy and Hunter, 1997; Mishra *et al.*, 2009). According to Connan *et al.* (2007) dose level of ^{210}Po accumulated via seafood intake is higher than ^{137}Cs and ^{90}Sr. An increase in ^{210}Po accumulation in individuals who consume protein-rich diets has also been reported (Watson 1985).

Polonium is stated to have a high affinity for protein, thus enabling it to enter the food chain in significant quantities, especially in people who consume high-protein diets such as meat, fish or other seafood (Lee and Wang, 2013). A normal diet consisting of seafood (presumed to be exposed to ^{210}Po, ^{210}Pb, ^{40}K, ^{87}Rb, ^{226}Ra and ^{14}C) such as fish (600 g), crustaceans, molluscs and seaweeds (100 g each) can provide an annual dose of 2 mSv. Of this dose, nearly 75% of the contribution is from ^{210}Po (Pentreath and Allington, 1988).

Dietary habits of sea fauna can also play a role in accumulation of polonium. Various fish species and their relation to dietary habits such as herbivores, carnivores or omnivores has been investigated by Hassona *et al.* (2008) who calculated ^{210}Po doses to be 4.14, 3.47 and 4.81 µSv year^{-1}, respectively.

According to Yamamoto *et al.* (1994), concentration of ^{210}Po and ^{210}Pb is minimal in meat and milk products, average in vegetables and cereals and highest in marine organisms. Many research reports are available from different parts of the world (e.g. Çatal *et al.*, 2012; Lee and Wang, 2013) relevant to detection of ^{210}Po at varied levels in the seafood and marine environment. Uddin *et al.* (2012) reported variations in ^{210}Po accumulation in different fishes (from Northern Arabian Gulf, within Kuwait's territorial waters) with different feeding habits. Their results revealed the highest concentration of ^{210}Po in fishes feeding on algae, zooplanktons and detritus (3.30 Bq kg^{-1}) with low concentrations detected in large carnivorous fishes (0.089 Bq kg^{-1}). This is a clear indication that ^{210}Po can be absorbed easily by phytoplankton and micro-zooplankton and thus become transferred via the marine food chain. Khan and Wesley (2011) reported the effect of ^{210}Po and ^{210}Pb in seafood and exposure and risk to humans from a nuclear power plant construction site (Kudankulam coast in India). They observed ^{210}Po concentration in seafood within the range 1.2–248 Bq kg^{-1}, while ^{210}Pb was 1.1–14.8 Bq kg^{-1}. Authors have also reported on the lifetime cancer risk for the public to be in the range of 3.47×10^{-5} to 1.62×10^{-3} for ^{210}Po and 4.03×10^{-5} to 1.96×10^{-4} for ^{210}Pb.
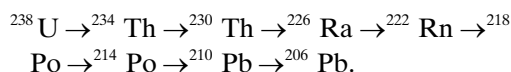
The activity values of ^{210}Po in fish from different seas and oceans have been reported as: Atlantic Ocean (Africa) (35 Bq kg^{-1}); Atlantic Ocean (South America) (0.8 Bq kg^{-1}); Indian Ocean (11 Bq kg^{-1}); Pacific Ocean (2.4 Bq kg^{-1}); Red Sea (33.5 Bq kg^{-1}); North Sea (57 Bq kg^{-1}); and Mediterranean Sea (4.8 Bq kg^{-1}) (IAEA, 1995). In addition, Musthafa and Krishnamoorthy (2012) reported high accumulations of ^{210}Po and ^{210}Pb (113.32 and 96.80 mBq L^{-1}) in *Perna viridis*, a bivalve mollusc, collected from Ennore Creek, South India. Based on this, authors have recommended that these molluscs be used as a bio-indicator of radionuclides. The concentrations of ^{210}Po and ^{210}Pb in fish samples from Turkey region (Aegean Sea) were reported as varying from undetectable levels to 499 Bq kg^{-1} and from 1.0 Bq kg^{-1} to 35 Bq kg^{-1}, respectively (Çatal *et al.*, 2012). These authors have also estimated highest dose contribution of ^{210}Po to humans to be 10,530 µSv year^{-1}. Recently, Lee and Wang (2013) reported ^{210}Po activity in oyster samples to be 25.9 Bq kg^{-1} (average), with an annual effective dose ingested among Taiwanese population via oysters to be 4.1×10^{-2} mSv year^{-1}. Daily intakes of ^{210}Po and ^{210}Pb via seafood (all in Bq day^{-1}) is reported to be 0.006–2.4×10^{-4} (Al-Masri *et al.*, 2000) in Syria; 0.48–0.69 and 0.02–0.04 in Japan (Yamamoto *et al.*, 1994); 91.6 and 117 in China (Quan *et al.*, 2008); and 0.27 and 0.00 in Poland (Skwarzec, 1997).

^{210}Po can easily enter lungs, kidneys and liver, but can be readily excreted in the faeces. It has been reported that 1 µg of ^{210}Po (with specific activity 166 TBq g^{-1}) ingested can deliver an effective dose of 40 Sv (Anonymous, 2010). According to the Nuclide Safety Data Sheet for 210-polonium, the exposure routes includes ingestion, inhalation, wound or via skin contamination, and the whole body is affected. The radiotoxicity of ^{210}Po is 5.14×10^{-7} Sv Bq^{-1} on ingestion and 2.54×10^{-6} Sv Bq^{-1} on inhalation (Nuclide Safety Data Sheet for 210-Polonium, assessed 14 July 2013).

These are just a few instances to highlight the serious problem the world is witnessing of bioaccumulation of ^{210}Po and ^{210}Pb via food.

15.7   Uranium, thorium and radium

Abnormal occurrences of uranium and its decay products in rocks and thorium in monazite sands are the main sources of the high natural background activity that has been identified in several areas of the world (Bolca *et al.*, 2007). Based on earlier studies by Sheppard *et al.* (1989), uranium is expected to be present as soluble complex of anions such as phosphates and sulphates and hence more available for uptake. Decay of U to Pb passes through various intermediate radioactive daughter nuclides emitting high-energy alpha particles at each stage. This scheme is reported by Ernst (2012):

$$^{238}\text{U} \rightarrow ^{234}\text{Th} \rightarrow ^{230}\text{Th} \rightarrow ^{226}\text{Ra} \rightarrow ^{222}\text{Rn} \rightarrow ^{218}\text{Po} \rightarrow ^{214}\text{Po} \rightarrow ^{210}\text{Pb} \rightarrow ^{206}\text{Pb}.$$

Among the various terrestrial radioisotopes, uranium and thorium can easily enter the human body via ingestion of foods (UNSCEAR, 2000). Monazite sand or rocks which are an orthophosphate of thorium and rare Earth elements comprises approximately 9% of thorium oxide and 0.35% uranium oxide (Fardeedudin and Sethna, 1958). Concentrations of U and Th in soil depend mainly on the soil type, parent rock, climate, vegetation and season (Shtangeeva *et al.*, 2005). Usually, ^{226}Ra or ^{228}Ra is stored as granules of calcium phosphate in mussel flesh (Ellis and Jeffree, 1982; Jeffree and Simpson, 1984). In fact, uptake rate of Radium has been reported to be inversely proportional to magnesium, calcium and water concentration (Jeffree and Simpson, 1986; Gerzabek *et al.*, 1998). A positive correlation has been reported between calcium and radium contents of shoot (Million *et al.*, 1994). Indeed, organic matter is reported to adsorb nearly 10 times the levels of radium as that of clay (Simon and Ibrahim, 1987). In addition, negative correlation between transfer factor of radium and pH has been reported by Gerzabek *et al.* (1998).

Generally, natural uranium can be detected in soil, water, air and in rocks and has three main isotopes: ^{234}U, ^{235}U and ^{238}U. Extensive use of

nuclear weapons, nuclear energy, coal combustion and phosphoric fertilizers are some of the contributors of U in the environment, which are all a result of human interventions (Tunney *et al.*, 2009; Carvalho and Oliveira 2010; Stojanovic *et al.*, 2011). High U contamination or concentrations (depleted, enriched or as natural in soil, water, vegetables and other foodstuffs) has been detected from various regions of the world (Bem and Bou-Rabee, 2004; Anke *et al.*, 2009; Gavrilescu *et al.*, 2009).

Absorption or uptake of U from plant varies among species and depends on the individual plant capacity to regulate uptake of the element (Hakonson-Hayes *et al.*, 2002). Information on the presence and transfer of U between soil, water and plants is necessary to identify their effects on public health (Takeda *et al.*, 2006). Apart from in plants, U has been detected in various foodstuffs screened from the US and the UK, with highest concentrations recorded in shellfish (9.5–31 μg kg^{-1}; EFSA, 2009). Many reports are available on the detection of high U in plants (such as in sunflower, Indian mustard, maize, lettuce, mushrooms) from the vicinity of uranium mining regions and with regard to phyto-remediation studies undertaken in the uranium-contaminated soil and water (Dushenkov *et al.*, 1997; Huang *et al.*, 1998; Neves *et al.*, 2008; Anke *et al.*, 2009).

Human diseases or health-associated risks due to the presence of U in the environment and ingestion include neurological and reproductive disorders, kidney failure (lesions and malfunction) and cancer (Bellés *et al.*, 2007; Zamora *et al.*, 2009; Arzuaga *et al.*, 2010; Leuraud *et al.*, 2011). The absorption of U in the human gastrointestinal tract depends on dose and solubility, and can fall within the range 0.1–6% of the ingested dose (EFSA, 2009). Nearly 41% of the U ingested by man is mainly via water or beverages, as estimated by Anke *et al.* (2009). In addition, 33% of U intake was via vegetables and 26% by consuming animal foods. Various levels of daily dietary intakes of U have been estimated worldwide (1 μg day^{-1} in India; 2.9–4.8 μg day^{-1} in Italy; 2.34 μg day^{-1} in Japan; Galletti *et al.*, 2003; Iyengar *et al.*, 2004; Ohno *et al.*, 2010).

According to Stojanovic *et al.* (2011), people residing near uranium mills or mines can be exposed to high uranium levels, especially from vegetables. The World Health Organization (WHO) has set up a tolerable daily intake limit of 0.6 μg kg^{-1} of body weight per day for uranium (WHO, 2005). In addition, United States Environmental Protection Agency has established a reference dose of 0.003 mg kg^{-1} day^{-1} for chronic oral exposure to U (IRIS, 2008).

The presence of uranium and ^{226}Ra has been reported in native apples (*Eugenia* sp. 0.04–0.37 Bq kg^{-1} fresh weight of U and 0.15–15 Bq kg^{-1} fresh weight ^{226}Ra) and native figs (*Ficus henneana* 0.07–0.19 Bq kg^{-1} fresh weight of U and 1.1–1.8 Bq kg^{-1} fresh weight ^{226}Ra) (Davy and Conway, 1974). Oufni *et al.* (2011) reported the absorption of ^{238}U and ^{232}Th in different parts of selected medicinal plants used to treat hypertension and diabetes in SE Morocco. Their study indicated that both the soil and plant parts contained higher U than Th. Results also revealed 43% of uranium (and thorium) was accumulated in roots, 26% in terms and 31% in leaves, but this depended on individual plant species studied.

Concentrations of various radionuclides (such as ^{238}U, ^{226}Ra, ^{210}Pb, ^{232}Th and ^{228}Ra) in different food categories such as grains, leafy vegetables, sugar, roots, fruits, meat, milk and milk products and fish have been throughly studied from the thorium-rich (monazite-bearing) region of Buena, Brazil (da Costa Lauria *et al.*, 2012). Exposure and detection of uranium, radon and other radionuclides in water and rocks from Lahontan Valley, Nevada was reported by Seiler (2012). Heavy metal concentrations (in cereal grains) created by local inclusions of U-mined soils (in the Ronneburg district, Germany) into non-contaminated cropland has been reported by Gramss and Voigt (2013). High levels of radioactivity were reported in soil of western Serbia: ^{238}U 60.4, ^{226}Ra 33.2, ^{232}Th 49.1, ^{40}K 379 and ^{137}Cs 36.4 Bq kg^{-1} (Dugalic *et al.*, 2010). Uranium bioconcentration has been reported in apple snail tissues (via freshwater bodies) and restricting of the consumption of ampullariid snails as human

and animal food has been recommended (Vega *et al.*, 2012).

Along with members of ^{238}U and ^{232}Th decay series, ^{40}K is also one of the predominant natural radionuclides present in nature. Monitoring ^{40}K in foodstuffs is important as potassium is naturally present in the human body with a significant role in cellular mechanisms (at levels of *c.* 0.14 kg). It is estimated that the general world population receives 180 μSv year^{-1} from ^{40}K (Khan *et al.*, 1995; Wang *et al.*, 1996). As the tetravalent forms of U and Th are particle reactive and insoluble in water, their absorption/movement via plant tissues is limited (Sheppard and Evenden, 1988). There are also many global reports detailing the presence of uranium in soil as well as its uptake by plants and estimated ingestion by humans (Kuwahara *et al.*, 1997; Yamaguchi *et al.*, 2007).

Thorium usually produces ^{220}Ra and actinium as the principal decay products. In nature, thorium occurs as ^{232}Th, a pure alpha emitter with a half-life of 14.05 billion years (Nuccetelli and Risica, 2008). Thorium has been successfully exploited for generating nuclear fuel, being a natural radionuclide encountered in the Earth's crust at concentrations of three times that of uranium. On ingestion, ^{232}Th easily accumulates in the bones (70%) and becomes deposited in the liver (4%). However, 10% is excreted in urine (ICRP, 1995). Various reports are available from India, Japan, Korea and Ukraine where high thorium content was detected in plant samples (vegetables and fruits) used as human food (Shiraishi *et al.*, 1997, 2000; Min-Soek *et al.*, 2008; Sathyapriya *et al.*, 2012). Martínez-Aguirre *et al.* (1995) reported that Thorium exhibits a much lower mobility than U, which is consistent with the results of Chen *et al.* (2005) who observed that Th has lower transfer factor values than U. Reports are available on the presence of ^{232}Th and its decay products in food and drinking water (UNSCEAR, 2000; Nuccetelli and Risica, 2008). The UNSCEAR (2000) report identified total internal exposure of terrestrial radioisotopes as 0.29 mSv (0.17 mSv from ^{40}K and 0.12 mSv from thorium and uranium series).

Breathing or inhaling radon and thoron gases as well as their progeny via uranium and thorium series can lead to exposure to internal radiation (Sathyapriya *et al.*, 2012). Inhaling thorium can increase the chance of developing lung diseases, as well as lung, bone and pancreas cancer. In fact, thorium can easily affect the genetic constituents, including the reproductive organs. Once inside the human body thorium accumulates in lungs, liver and the skeleton. The principal site of deposition of thorium in the body is the bones. In addition, thorium is deposited in the soft tissue (16%) and the gonads (1%).

Radium is a radioactive element (isotopes ^{226}Ra, ^{224}Ra and ^{228}Ra) produced by the decay of uranium and thorium in the environment. It is present naturally in rocks, soil and in water, as well as produced artificially by human interventions such as burning of coal and uranium mining. Radium finds its use in medicine for cancer treatment (as radon gas) and in luminous paint (in the form of radium bromide). Exposure to high levels of radium can lead to eye problems (cataract), tooth problems and cancer. ^{228}Ra can be a significant contributor to dose ingestion as its coefficient is three times higher than that of ^{226}Ra. Hence, the presence of this radionuclide in food and drinking water is a cause of concern. Phosphate ores of marine origin encompassing nearly 1200–1500 Bq kg^{-1} ^{226}Ra and other trace metals have been processed in Belgium for more than 50 years to obtain calcium phosphate to be used as cattle food, contributing to intake via contaminated pasture. The byproduct of this process includes wastewater discharged into rivers, thus raising concerns of contamination (Gao *et al.*, 2010). The presence of radionuclides in milk is attributed to grazing on contaminated pasture followed by ingestion of contaminated animal products. Presence of ^{226}Ra in cow's milk and other meat products has been reported (Kirchmann *et al.*, 1972; Watson, 1985).

The entire transfer process is referred to as the 'pasture-cow-milk exposure route' by Dinis and Fiúza (2007). These researchers developed a 'compartment dynamic model' to predict the concentration along this route. This model used

mathematical formulae relevant to radium behaviour along the exposure route, and is useful for predicting the activity concentration in each of the individual compartments.

Shanthi *et al.* (2012) reported the transfer factor (TF, concentration of radionuclide in a crop/soil to plant transfer) of long-lived radionuclides (such as ^{238}U, ^{232}Th and ^{226}Ra) in various food crops from India. Their results showed that non-edible parts accumulated more radionuclides than the edible parts in fruits and vegetables. Tufail *et al.* (2010) reported an annual effective dose received from natural radioactivity (^{40}K, ^{226}Ra and ^{232}Th) intake through wheat grain produced in Pakistan of 217 μSv.

15.8   Other radionuclides in food

There is still a scarcity in data on the concentrations of Strontium (^{90}Sr) and Cesium (^{137}Cs). One of the most in-depth studies undertaken includes that of Landstetter *et al.* (2013), who reported on the consumption pattern of various foodstuffs (minerals and drinking water, milk, wild boar, deer, beef, pork, cheese, mushroom and mixed diet) and ingestion dose received from ^{137}Cs, ^{90}Sr and ^{40}K .Accordingly, their results showed ingestion dose of ^{137}Cs and ^{90}Sr to be <1%.

Strontium is a natural non-radioactive element, while its isotope radio-strontium or strontium-90 (half-life of 29.1 years) is radioactive. ^{90}Sr is a byproduct of fission of uranium and plutonium. Apart from ^{90}Sr, ^{89}Sr and ^{85}Sr can be distributed in the environment where they find use in nuclear reactors, and as a tracer element used in agricultural studies and medicine. However, nuclear accidents also render the presence of ^{90}Sr in the environment. The best examples are those of Chernobyl and Fukushima nuclear accidents. Reports available on the presence of ^{90}Sr in food is scarce due to stringent safety laws imposed in and around nuclear reactors. ^{90}Sr has an identical structure to that of calcium and hence can be easily accumulated in bones, teeth and soft tissues (in humans) leading to bone cancer and leukemia. Monitoring of food or drinking water after any nuclear accidents is therefore of high importance. The presence and release of low levels of ^{90}Sr from the Fukushima reactors after the accident as well as their presence in soil samples and vegetation was reported by Steinhauser *et al.* (2013). Their results indicate the intrinsic coexistence of ^{90}Sr with ^{137}Cs with ^{90}Sr contamination levels not exceeding the limits (maximum 10% of ^{90}Sr activity to that of respective ^{137}Cs activity). The co-existence of the two radionuclides in the environment has been recommended to be of significance in the current food monitoring campaigns.

Cesium-137 or radio-caesium is formed as a nuclear fission product of ^{235}U in nuclear reactors. ^{137}Cs can easily spread in the environment, has a high water solubility rate and forms caesium hydroxide. The half-life of ^{137}Cs is 30.17 years, with the biological half-life being nearly 70 days. ^{137}Cs has been commonly used in food irradiation and nuclear medicine for treating cancer. However, on ingestion, it can easily accumulate in the human body (via intake of food or water), especially in bones and muscle tissues. The most recent report on ^{137}Cs in food is by Peters *et al.* (2013). These researchers evaluated various food products (coconuts, pandanus, breadfruit) from residence islands of Bikini, Rongelap Atolls, Enewetak and Utrōk Atolls in Marshall Islands and found activity to be higher in copra meat followed by pandanus, breadfruit, copra juice, drinking coconut meat and drinking coconut juice.

Prior to the Fukushima incident, studies were performed in Japan during 2003–2005 (Sugiyama *et al.*, 2008) to detect concentrations of ^{137}Cs in imported foods which included agricultural products, livestock products, marine products and other food. Accordingly, concentrations of ^{137}Cs were within the range 0.04–156 Bq kg^{-1}. Harada *et al.* (2013) have recently reported dietary intake of radio-caesium among the adult residents in Fukushima prefecture and neighbouring regions since the Fukushima nuclear accident. They analysed 53 sets of duplicate food samples (of 24 hr). Based on the results, radio-caesium was present in 25 out of a total 26 samples from Fukushima

region with high variations in ^{134}Cs (<0.20–7.7 Bq day^{-1}) and ^{137}Cs (<0.26–9.7 Bq day^{-1}) recorded. The average radioactivity for ^{134}Cs and ^{137}Cs in persimmons and apples (local produce) were 23 and 30 Bq kg^{-1}, respectively. Rosén *et al.* (2011) reported that a single treatment (100 kg K ha^{-1}) of forest soil with potassium chloride fertilizer in 1992 had the long-term effect of 17 years on the bioaccumulation of ^{137}Cs.

15.9   Minimizing internal exposure by ingestion after long-scale radiation releases

As in any other case of chemical accidents, experts have recommended certain measures aimed at reducing exposure by ingestion in the case of future nuclear accidents. Accordingly, limiting the consumption of food and water around the accident sites, temporarily banning marketing and consumption of selected foods and setting up maximal frequency (or permeable limits) of consumption can be helpful. For example, the Swedish authorities had recommended restraining fish consumption from areas where the Chernobyl fallout caused ^{137}Cs activity concentrations above 300 Bq kg^{-1} to about once per week for the 300–1500 Bq kg^{-1} range and a few times per year when the value was >1500 Bq kg^{-1} (Bruce and Slorach, 1987). Other measures include the supplementation of iodine to counteract the accumulation of ^{131}I in the thyroid gland, especially in young children (Cardis *et al.*, 2005), avoiding grazing of cattle in contaminated areas (Bruce and Slorach, 1987) and washing vegetables with pressure-jet water which can reduce the radionuclide concentration (Muramatsu *et al.*, 1987; Wilkins *et al.*, 1987; Constantinescu *et al.*, 1988). This effect has been attributed to the washing out of ^{131}I combined with dust in leaves (Muramatsu *et al.*, 1987). In addition, cooking or boiling in mild salted water has been reported to remove nearly 70% of ^{131}I in vegetables (Muramatsu *et al.*, 1987) and 80% of radio-caesium in meat (Lofti *et al.*, 1990). Treatment of mushrooms with acetic acid (2%)

has been shown to significantly decrease radio-caesium activity (Dvořák *et al.*, 2006).

15.10   Conclusions and future outlook

Based on the available reports and considering the present scenario, there is an urgent need to evaluate the presence of radionuclides in our environment and their concentration in food. Soil and water remediation and safe disposal of radioactive waste are imperative to minimize pollution by radioactive nuclides. Disposal of radioactive wastes should follow stringent international laws and regulations. As radionuclides are ubiquitous in nature, research work needs to be expanded in all the regions where its presence has not yet been determined, which can be either in flora or fauna of land or aquatic biome, especially around mining areas. As plants can easily take up naturally occurring nuclides, bio-indicator plants should be introduced (planted) in and around the suspected areas and geographical profiling should be performed. The database from international governing bodies should be updated regularly and made available for the general public in a user-friendly format.

Detailed investigations on the presence and bioaccumulation of radionuclides in terrestrial and aquatic animals and their tissues are necessary (especially on uptake mechanisms involved), as some of these animals are also an integral part of the human food chain. The majority of the research reports have not examined the presence of radionuclides in animal feed or diets, which is expected to provide vital information about transfer along the food chain. It is suggested that daily intake levels of radionuclides be monitored on a regular basis among local populations where potentially contaminated local or imported food products are consumed. Research work has been undertaken on selected radionuclides, and requires to be expanded for all the others such as for ^{137}Cs and ^{90}Sr. In addition, the majority of work undertaken is on seafood since they are easily available. However, it

would be interesting to derive radionuclide concentrations of deep-sea flora and fauna. Data on wild and underutilized plants used as food by tribal populations are also scarce, which requires further investigation. In addition, sample collection, preparation and transporting from sampling sites should be standardized with the same method (uniformity in sampling), applicable worldwide.

New ready-to-use kits or other rapid detection instruments and methods need to be developed for screening of radionuclides in human foodstuff and livestock feed. These instruments should be economical, efficient, reliable and easy to handle and ought to effortlessly respond during radiation emergency. There is still a scarcity of information on the possible long-term health effects of consumption of radioactive-contaminated foods at low levels, which is definitely of interest. These informations could be useful to instigate efficient waste management schemes. Following enhanced demand for organically grown fresh produce, there is a scarcity of information generated on the presence of radionuclides in such produce which must be addressed in the near future.

Finally, the question that needs to be answered is whether the present world is ready and prepared for another nuclear accident. If no, then how will safe foods be provided during times of emergency? Although the presence of natural radionuclides in the environment and hence in foodstuffs is unavoidable, anthropogenic interference with nature contributing to the presence of radionuclides must be monitored and managed.

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16
Antinutrients and Toxicity in Plant-based Foods: Cereals and Pulses

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Summary

Apart from being a good source of proteins, the majority of pulses are rich in naturally occurring micronutrients. In addition, they encompass antinutritional factors which play vital physiological and metabolic roles in human health. Due to their health benefits, these micronutrients are presently marketed as functional food ingredients. Cereal grains may protect the body against age-related diseases including cardiovascular ailments, cancers and diabetes, possibly as a result of their dietary fibre content and the presence of micronutrients in the outer layers and germ fractions of a grain. Cereals also provide minerals and vitamins such as iron, zinc, copper selenium, manganese and vitamin E, folates, phenolic acids, phytic acid, lignins, lignans and alkylresorcinols. Under *in vitro* and *in vivo* conditions, phenolic acids such as ferulic contain free-radical oxygen scavenging capacities. The antioxidant property of cereals and cereal products is enhanced during digestion and creates a favourable antioxidative atmosphere which is beneficial for the epithelium tract in the large intestine. Various processing techniques such as soaking, boiling and steeping in water may be applied to reduce the toxic factors from cereals and pulses.

16.1   Introduction

Basic foods consumed by humans are a complex mixture of various naturally occurring chemical compounds which provides colour, aroma, taste and texture in addition to provision of nutritional elements. Naturally occurring intrinsic chemicals of food above threshold level may be hazardous or even fatal to the consumer. Hazard description in the discipline of food safety endeavours to appraise negative health effects of compounds that may be present in food. The benchmark dose (BMD) approach is applied in the food risk assessment because it offers several benefits compared to the unadventurous no-observed-adverse-effect-level approach. The BMD approach is a standard applicable to mycotoxins, pesticides and natural toxins present in foodstuff (Muri *et al.*, 2009). For instance, an excess of vitamin A may cause anorexia, headache, vomiting, peeling of skin and swelling of long bones, while hideously increased intake of vitamin D may result in vomiting, diarrhoea, weight loss and kidney damage. Intakes of some essential minerals in excess quantities are also responsible for

toxicity and health hazards (Table 16.1). It seems that most foods contain one substance or another whose accumulation in the body may prove harmful.

To protect from possible hazards from the predators, plants have self-protective mechanisms. Examples include plants with thorns for their protection, offensive odours or toxic chemical compounds in their tissues. For instance, the lectins of black locust (*Robinia pseudoacacia*) and elderberry (*Sambucus nigra*) bark cause the same severe toxicity symptoms as *Phaseolus vulgaris* agglutinin (PHA). Since bark lectins are abundant, elderberry and black locust are never attacked by rodents, deer or other wildlife, whereas the bark of surrounding lectin-free species including poplar, willow and wild apple is favourite food for the same animals (Colegate *et al.*, 1998; Than *et al.*, 2005).

Several thousands of plants have been used by man as food, out of which only a handful have been selected for domestication. In this process, several plants have lost some of their defensive mechanisms and appear to have been tamed as food plants in a similar way to domestication of

Table 16.1 Mineral elements, their sources and possible harmful effects due to excess in diet. Source: Awan and Anjum, 2011. Reproduced with permission of the authors.

Element	Source	Toxic effects
Aluminium	Citrus fruits, stone fruits, cooking utensils	Gastrointestinal irritation, rickets
Arsenic	Sea foods	Nausea, vomiting, diarrhoea, burning of mouth and throat, severe abdominal pains
Copper	Drinking water, cooking utensils, some permitted colours, preservatives	Hepaticolenticular degeneration (Wilson's disease)
Iron	Liver, kidney, heart, legumes, shellfish	Haemochromatosis, enlargement of liver, pigmentation of skin, diabetes mellitus, cardiac failure
Lead	Drinking water, food containers, cigarette smoking, vehicle exhaust, bones	Abdominal colic, encephalopathy, myelopathy, peripheral neuropathy, anaemia
Phosphorus	Milk, dairy products, meat, poultry, grains	Erosion of jaw
Sodium	Common salt, several foods	Nausea, vomiting, in hypertension leads to oedema
Tin	Widely distributed in foods, tin cans	Vomiting
Zinc	Wheat germ, bran, oysters, drinking water	Irritability, stiffness and pains in the muscles of back and neck, loss of appetite, some nausea, vomiting, diarrhoea

wild animals. Despite this, there still remain intrinsic chemical compounds in some of these plants which can prove toxic to human beings at certain levels. Under normal conditions, when a variety of food is consumed in reasonable quantities, the danger of any hazardous effect becomes inaccessible (Neilsen and James, 1992). The human body has developed built-in mechanisms through which most intrinsic or other toxic substances are either converted into harmless compounds or are excreted from the body. The probability of toxicity by such substances is rare if a balanced diet is eaten in reasonable quantity and toxic substances are not allowed to accumulate in the body. Consequently, when normal healthy individuals eat foods containing these substances in normal amounts, there may not be any ill effects. However, when such foods are consumed in large quantities over an extended period, illness may result (Dorling *et al.*, 1993).

16.2   Toxicity

16.2.1   Accidental toxicity

In certain selected cases, accidental toxicity may occur from the consumption of foods of plant origin due to ignorance of a consumer. Wild arum (*Arum maculatum*), deadly nightshade (*Atropa belladonna*) and white bryony (*Bryonia dioica*) have caused poisoning, particularly among children who have mistakenly eaten their fruits. Accidental chewing of raw cashew nut irritates the skin and causes swelling. Consumption of fresh and poorly cooked cassava tubers of some varieties has proved fatal due to the presence of the cyanoglucosides linamarin and lotaustralin which are hydrolysed by endogenous enzyme linamerase to acetonecyanohydrin and cyanide (Nambisan, 2011; Saka and Nyirenda, 2012). Ackee apple (*Blighia sapida*) is a member of the Sapindaceae (soapberry family) and native to tropical West Africa. Eating unripe ackee fruit causes Jamaican vomiting sickness. The walnut-like aril is safe to eat only when fully ripe. The unripe fruit contains a water-soluble toxin, hypoglycin A (α-aminomethylenecyclopropylpro

pionic acid) and the less-toxic hypoglycin B (2S)-2-amino-4-(1S)-1-carboxy-2-[(1S)-2-methylidenecyclopropyl] ethyl] carbamoyl] butanoic acid. It causes sores in the mouth, drowsiness, vomiting, convulsions and coma. Consumption of untreated grasspea (*Lathyrus sativus* L.) over long periods causes neurolathyrism in animals and human beings. It contains toxic amino acid beta-N-oxalyl-alpha,beta-L-diaminopropionic acid (ODAP). The safe use of grasspea (*Lathyrus sativus* L.) and allied species (*L. cicera*, *L. clymenum* and *L. ochrus*) requires a better understanding of the factors that are involved in the development of neurolathyrism (Barennes *et al.*, 2004; Barceloux, 2009a; Enneking, 2011).

16.2.2   Toxic compounds in legumes and cereal grains

Irrespective of the health benefits of pulses, their consumption in Western societies is customarily very low. This is partly due to the perception that pulses are a source of flatulence and gastrointestinal distress. They contain oligosaccharides which are well tolerated, with trifling perceived changes in flatulence and gastrointestinal upset. Toxicity from the consumption of legumes occurs due to the presence of antinutritional factors such as protease inhibitors, haemagglutinins, phytic acid, tannins, cyanogens, lathyrogens and allergens (Maia *et al.*, 2000; Preet and Punia, 2000; Enneking, 2011). Other example are two millet species, fonio and pearl millet, which have been cultivated for food in sub-Saharan Africa and India. High consumption of these two species is known to cause goitre due to the flavonoids apigenin and vitexin in fonio and pearl millets, respectively. These are strong inhibitors of thyroperoxidase (TPO) (Hertzel, 1989; Gressel, 2008).

16.3   Plant-derived allergens

Some common allergens are widely present in plant foods and the consumer might be unaware of their presence until facing their toxic

effects. Their minor quantities may cause a life-threatening incident known as an anaphylactic reaction (Morandini 2010). This may occur on skin contact, injection or inhalation and ingestion of an allergen. In the UK alone, allergens in food have been reported to cause 48 deaths over a period of seven years from 1999 to 2006 (Pumphrey and Gowland, 2007). Half of the eight foods accounting for 90% of all food-allergic reactions (fish, shellfish, milk, egg, tree nuts, peanut, soybean and wheat) are of plant origin (Clare Mills and Shewry, 2005). The transgenic plants have never caused allergic reactions to consumers, contrary to common perception. In one case, a gene for 2S albumin from the Brazil nut, which is a known nut allergen, was expressed in soybean. This transgenic soybean has been tested for allergic reaction, and it was found that the 2S albumin is really a major Brazil nut allergen (Nordlee et al., 1996). In the US and Europe, 5–8% of babies and 2% of adults are allergic to soybeans. The chief soybean allergen is a protein and sensitization to the soybean allergens Gly m 5 (β-conglycinin) or Gly m 6 (Glycinin) is potentially indicative of severe allergic reactions. All subunits of Gly m 5 and Gly m 6 are IgE-reactive: 53% of subjects have specific immunoglobulin-E (IgE) to at least 1 major storage protein, 43% to Gly m 5 and 36% to Gly m 6. Gly m 5 is found IgE-reactive in 5 out of 5 and Gly m 6 in 3 out of 5 children. IgE-binding to Gly m 5 or m 6 is found in 86%. Breeding and mutagenesis practices have permitted the depletion of some soybean allergens (Ogawa et al., 2000; Holzhauser et al., 2009).

A similar state of affairs is found for transgenic peas expressing the bean α-amylase inhibitor (Prescott et al., 2005). The transgenic peas dragged out an immune response in mice upon feeding. The reaction could be attributed to changes initiated in the plant by the regeneration and conversion process or by the changes perceived in the α-amylase inhibitor between peas and beans concerning glycosylation pattern and the depletion of amino acid residues of the protein (Chen et al., 2009).

16.3.1 Haemagglutinins, trypsin and protease inhibitors

The haemagglutinins and trypsin inhibitors are proteinaceous compounds present in numerous legumes such as soybeans, mung beans, kidney beans, chickpeas, cowpeas, and lentils as well as in some cereals. They cause poor food utilization and impair growth of the individual. Their concentration varies depending on type and variety of beans. In cowpeas (V. unguiculata), haemagglutinating activity is higher in the albumin fraction and varies from 30,900 to 444,400 HU kg^{-1} flour (Vasconcelos et al., 2010). Soybeans contain an antitryptic factor; this trypsin inhibitor is responsible for the poor digestibility of this useful legume. These toxic compounds can be minimized by applying a series of operations such as soaking, germinating, steaming and fermenting (Ndubuaku et al., 1989; Radha et al., 1989).

Fava beans (Vicia faba, also known as broad beans, horse beans and Windsor beans) are liable to cause a disease known as favism, especially in the Mediterranean region. This disease is characterized by haemolytic anaemia, haemoglobinuria and jaundice, often accompanied by high fever. The disease can start within a few hours after consumption of the beans. Although complete recovery in adults may start after 24–48 hours of the illness, it may cause death in children. The disease is ascribed to an instinctive disorder in metabolism; susceptible individuals have a deficiency of the enzyme glucose-6-phosphate dehydrogenase in the erythrocytes. Moreover, glycoproteins that agglutinate erythrocytes of some or all blood groups in vitro depend on their specificity and high binding affinity for a specific carbohydrate moiety on the cell surface (González de Mejía et al., 2002). Beans (Phaseolus vulgaris) are considered important sources of lectins. Kidney bean phytohemagglutinin (PHA) is a tetrameric glycoprotein consisting of two different subunits with a molecular weight of c. 120 kDa (Sgarbieri and Whitaker, 1982).

Based on molecular weights and cystine contents, protease inhibitors isolated from legumes are categorized into the families Kunitz and

Bowman-Birk. Kunitz have a molecular weight of *c*. 20 kDa, two disulphide bridges and are found active specifically against trypsin, while Bowman-Birk have a molecular weight of 8–10 kDa with seven disulphide bridges. These have the ability to inhibit trypsin and chymotrypsin simultaneously at independent binding sites. The protease inhibitors of lima beans, cowpeas and lentils are characterized as members of the Bowman-Birk family (Belitz and Weder, 1990; Liener, 1994). Trypsin/chymotrypsin inhibitors of Brazilian pink bean, red kidney bean, soybean and lima bean are closely associated with high homology (Wu and Whitaker, 1991).

Pulse lectins are important tools in cell biology and immunology, with potential for clinical applications. Legumes contain considerable quantities of lectins ranging from around 0.6% of the total protein (24–25%) in garden peas, 2.4–5% of the total protein (17–23%) in kidney bean seeds to 0.8% in lima bean and soybean protein (21% and 34%, respectively). Common beans contain a higher content of trypsin inhibitors and lectins than tepary beans (Zhang *et al*., 2009). Grant *et al*. (1995b) determined trypsin inhibitor and lectins in cowpeas, kidney beans, lupin seeds and soybeans. High amounts of lectin in kidney beans (840×10^{-5} hemagglutinating activity units or HU kg^{-1}), while very low content in lupin and cowpea seeds (3×10^{-5} HU kg^{-1}) were determined. The quantity of protease inhibitor is moderate in cowpeas and kidney beans such as 10.6 g and 8 g of trypsin and 9.2 g of chymotrypsin inhibited kg^{-1}, respectively, and low in lupin seeds (1.1 g of trypsin and 1.4 g of chymotrypsin inhibited kg^{-1}). However, Brazilian bean varieties with different seed coat colours have high trypsin inhibitory activity (TIA) of 18–29 mg^{-1} (Lajolo and Genovese, 2002).

16.3.2 Goitrogens

Some of the natural chemical compounds such as goitrogens are compounds that obstruct the role of the thyroid gland in making its hormone. If the thyroid gland has difficulty in the production of thyroid hormone, it may enlarge to compensate

insufficient synthesis of hormone. Goitre is the most apparent symptom of iodine deficiency; however, reproductive failure, mental retardation, brain damage and childhood mortality are more severe consequences. There are annotations that show the existence of factors other than iodine deficiency, however. Observations reveal that a number of plant foods usually consumed by the people of several regions contain goitrogenic/antithyroidal compounds that interfere with thyroid hormone synthesis, acting at different levels in the thyroid gland and causing goitre and associated iodine deficiency disorders (Chandra, 2010). Among the commonly used vegetables, members of the Cruciferae family, especially species of the genus *Brassica* such as cabbage, turnip and radish, contain an appreciable amount of toxic substances collectively known as glucosinolates. Concentration of glucosinolates is normally higher in seeds than in other parts of a plant. On hydrolysis with suitable enzymes, these substances yield goitrins (antithyroid agents) which have potent goitrogenic activity. Sporadically, consumption of large quantities of cabbage or kale over an extended period has been responsible for goitre. Cows feeding on such commodities secrete goitrins in milk which in some instances have caused goitre in children. The goitrogens are thioglycosides which are responsible for enlargement of the thyroid, a disease caused mainly by the deficiency of iodine in the diet (Rosa, 1997).

16.3.3 Cyanogens

Cyanogens are cyanogenic glycosides and are present in peas, beans, pulses, fruit kernels and cassava. They cause hydrogen cyanide (HCN) poisoning. Toxicity from the consumption of cassava (*Manihot sp*.) is common among several African and Latin American countries due to its high levels of production and consumption. Consumption of cassava roots and leaves which contain huge quantities of cyanogens may cause cyanide poisoning, with symptoms of headache, nausea, dizziness, diarrhoea and vomiting, sometimes leading to death. Acute toxicity results in

convulsions, paralysis and cessation of respiration, followed by death. Chronic toxicity causes two important diseases, namely tropical toxic neuropathy and endemic goitre (Nhassico *et al.*, 2008).

The cause of toxicity of cassava is due to the presence of the cyanoglucosides linamarin and lotaustralin which are hydrolysed by endogenous enzyme linamarase to acetonecyanohydrin (ACN) and cyanide (CN). For the reduction of cyanoglucosides, research efforts are focused on: (1) processing techniques to remove cyanogens; (2) controlling its metabolism; and (3) development of acyanogenic cassava varieties by breeding. Its content in cassava is genetically exploited and cultivars may be grouped as low (<50 μg g^{-1}), medium (50–100 μg g^{-1}) and high CN (>100 μg g^{-1}) varieties.

Molecular techniques for reducing tuber cyanoglucosides (CNG) have focused on development of transgenic plants with reduced expression of cyt P 450 in leaves, or increased expression of hydroxynitrilelyase in tuber. Traditional methods used for processing are boiling, drying, parboiling and drying, baking, steaming, frying and preparation of flour (Coursey, 1973). Reductions in CN of 25–98% have been observed by applying these processes. In order to attain safe limits of 10 μg g^{-1} in cassava products, new methods of processing, especially for cassava containing more than 250 μg CN eq g^{-1}, remain a challenging problem (Nambisan, 2011). Total cyanogen reductions of 97.9 ± 0.5% and 82.4 ± 1.0% may be acquired upon soaking peeled and unpeeled roots, respectively. The residual cyanogen content (2.6 ± 0.7 mg HCN eq kg^{-1}) in flour produced from peeled roots is lower than the FAO/WHO limit (10 mg HCN eq kg^{-1}). Flour from the unpeeled method has 20 times more residual cyanogen (53.8 ± 1.8 mg HCN eq kg^{-1} dry matter). The peel contains four times higher cyanohydrin than the pulp.

Cassava tubers are known to contain appreciable quantities (average 38 mg 100 g^{-1}) of some cynogenic glycosides (linamarin and lotaustralin) which, upon treatment with acid or appropriate hydrolytic enzymes, yield hydrogen cyanide (HCN), a deadly poison. The lethal dose of HCN for an adult person ranges from 0.5 to 2.5 mg kg^{-1} body weight. The level of HCN is drastically reduced when cassava tubers are processed into 'gari'. The amount of HCN present in gari usually varies from 1.0 to 2.0 mg 100 g^{-1}, being higher in the village-produced products (Saka and Nyirenda, 2012).

Cyanogenic glycosides are also present in many fruit pits including apricot kernels. Toxicity may be caused by the consumption of large amounts of apricot kernel pits. When in contact with stomach acids, the cyanogenic glycosides release cyanide, which is an active component. In the northern areas of Pakistan, where apricot is available in abundance, the residents save the pits and during winter use the shells as fuel and eat the kernels. There is a likelihood of prevalence of apricot toxicity in these areas. In 1993 apricot kernels imported from Hunza Valley, Pakistan into the United States were recalled due to the presence of excessive amounts of the toxin (Suchard *et al*, 1998).

The cyanogenic glycoside linamarin, present in several varieties of Lima beans, is often leached into water during soaking and boiling. Moreover, the enzymes responsible for hydrolysis of toxic compounds are destroyed during cooking, thereby preventing the release of HCN. Any cyanogenic toxicity that may occur from the consumption of such beans is often due to inadequate soaking and cooking. Although boiling destroys the enzyme responsible for hydrolysis of linamarin, it does not necessarily detoxify a cyanogenic glycoside (Dibofori *et al.*, 1994).

16.3.4   Lathyrogens

Lathyrogens are nitriles found in chickling vetch and chick peas ('khesri dal', 'matri') and are responsible for skeletal deformities and damage to the central nervous system (neurolathyrism). Several species of the genus *Lathyrus* (e.g. *L. sativus*, *L. cicera*, *L. clymenum*) which are habitually consumed in India, Algeria and certain other areas contain high concentrations of toxic nitrogenous compounds collectively known

as lathyrogens. The toxic constituents are nitriles which contain –C Ξ N functional group. *Lathyrus sativus* L. (Vetch) is a resilient subtropical/tropical legume crop which is also known as grass or Indian pea. Beans of this so-called 'famine crop' are a chief source of nutrition among poor people in Africa and Asia. Its seed contains the neurotoxin β-N-oxalyl-L-α-β-diamino-propionic acid (BOAA) which causes a disease known as lathyrism, a paralysis of lower limbs in both men and animals. It is widespread among adults in Central India who consume it in large quantities (above 33% in the diet) for 3–6 months. However, in extreme cases it may cause death. When consumed as a supplement to an otherwise adequate diet, it does not produce the toxic effects (Enneking, 2011).

Safe content for BOAA is <0.2%, while it ranges between 0.3 and 3.3% in germplasm (Mustafa *et al.*, 2007; Barceloux, 2009b). Soaking and boiling of grains decrease BOAA levels but nutritional quality is decreased as a consequence of efficient detoxification. However, orthodox breeding and tissue culture techniques have already produced varieties with significantly reduced BOAA levels (Vaz Patto *et al.*, 2006). By extensive outcrossing for this crop, low BOAA levels can be produced and provided to farmers every year (Yadav and Bejiga, 2006).

16.3.5   Lignins and lignans

Lignin represents 30% of plant biomass. They are the foremost component of wholegrain cereals and may constitute 3–7% of the bran fraction. They were long ago known to be nutritionally inert in the digestive tract; nonetheless, their polyphenolic structures show impending antioxidant capacities. Begum *et al.* (2004) reported that rats can metabolize lignins into mammalian lignans. Although it is reasonable to suppose that lignins have antioxidant effects *in vivo*, predominantly in the colon, nearly all investigations on their antioxidant potential are performed *in vitro* (Reinosa *et al.*, 1998) or on their effect on DNA damage (oxidative lesions) in isolated mammalian cells

(Slamenova *et al.*, 2000; Labaj *et al.*, 2004). Consequently, diabetic rats (diabetic nephropathy) given subcutaneous injections of nordihydroguaiaretic acid for 4 weeks demonstrated less renal dysfunction and oxidative stress such as lipid peroxidation, antioxidant enzymes, SOD, glutathione and catalase than controls (Anjaneyulu and Chopra, 2004).

Lignans are dietary phyto-oestrogens that are present in a wide variety of plant foods including whole cereal grains such as corn, oats, wheat and rye and flax seeds. The group includes matairesinol, secoisolariciresinol, pinoresinol, syringaresinol and lariciresino. They have a polyphenolic structure and may have antioxidant properties. The lignans and their metabolites such as mammalian lignans enterodiol and enterolactone have antioxidant activity in lipids and aqueous *in vitro* model systems and decrease lipid oxidation (Kitts *et al.*, 1999). The lignans, diphenolic compounds with a 2,3-dibenzylbutane skeleton, have both estrogenic and antiestrogenic properties (Orcheson *et al.*, 1998). The plant lignans, secoisolariciresinol (SEC), and matairesinol (MAT) are converted to the metabolites enterodiol (ED) and enterolactone (EL), known as the mammalian lignans, in the gastrointestinal tract.

The concentrations of lignans in legumes is within the range 0–240 g 100 g^{-1} for SEC with higher values for oilseeds, peanuts (333 g 100 g^{-1} SEC) and soybean (13–273 g 100 g^{-1} SEC) with trace or no MAT (Mazur *et al.*, 1998). The lignans have less obvious influences than lignins upon oxidative genetic damage, as observed in human colon cells which are incubated with enterolactone. Furthermore, healthy postmenopausal women who ate a low-fat muffin enriched with a lignan composite for 6 weeks demonstrated no evidence of change in their serum lipoprotein oxidation resistance or plasma antioxidant capacity. On the other hand, young rats fed flax and lignan secoisolariciresinol diglycoside showed that the lignan metabolites enterodiol and enterolactone have an antioxidant sparing effect (Yuan *et al.*, 1999; Pool-Zobel *et al.*, 2000; Hallund *et al.*, 2006).

16.3.6 Phytate

Phytates are prime storage forms of both phosphate and inositol in plant grains mainly present in the bran fraction of wholegrain cereals, especially within the aleurone layer. It comprises 1–3% of cereal grains, nuts and legume seeds and is also present in small quantities in tubers, roots and vegetables. Wholegrain cereals and legumes are high in phytate content and minerals such as Zn, Fe and Mg (Sandberg, 2002). In legumes, it is found in the protein bodies in the endosperm as a mineral complex, which is insoluble at the physiological pH of the intestine (Fredlund *et al*., 2006).

The phytate and its degraded products are well-known inhibitors of absorption of essential dietary minerals, particularly Zn and non-haem iron. It forms complexes with dietary minerals and causes mineral-related deficiency in humans. It also hinders the lipid and protein utilization in the body (Hurrell *et al*., 1999). However, the absorption of minerals depends on the composition of consumed food. In a balanced diet containing animal protein, a high intake of legumes does not entail a threat of inadequate supply of minerals (Sandberg, 2002). Moreover, phytic acid is a most important phosphorus storage compound in legumes and cereal grains, contributing about 1–7% of their dry weight. It may contribute more than 70% of the total grain phosphorus (Graf and Eaton, 1990; Zhou and Erdman, 1995).

In pulses, phytosterols are present in minute concentrations and the most common phytosterols are β-sitosterol, campesterol and stigmasterol (Benveniste, 1986). These phytosterols are also plentiful as sterol glucosides and esterified sterol glucosides, with β-sitosterol accounting for 83% of the glycolipids in defatted chickpea flour (Sanchez-Vioque *et al*., 1998). Total phytosterol content determined in the legumes ranges from 134 mg 100 g^{-1} in kidney beans to 242 mg 100 g^{-1} in peas. Total β-sitosterol content ranges from 160 mg 100 g^{-1} in chickpeas to 85 mg 100 g^{-1} in butter bean. Peas and chickpeas contain high levels of campesterol (25.0 mg 100 g^{-1} and 21.4 mg 100 g^{-1}, respectively).

Butter bean and kidney beans contain 86 and 41.4 mg 100 g^{-1} stigmasterol contents and peas contain 1.0 mg 100 g^{-1} squalene content (Ryan *et al*., 2007). However, phytosterol levels for kidney beans are 127 mg 100 g^{-1}, with much lower concentration of phytosterols for chickpeas (35 mg 100 g^{-1}) as opposed to 205 mg 100 g^{-1} in this study (Weihrauch and Gardner, 1978). In raw dry legumes, inositol phosphate (InsP) is present in various forms including InsP3 (28%), InsP4 (10%), (InsP5) 4% and (InsP6) 2% and in cooked dry legumes 8, 4, 2 and 2%, respectively. Raw lentils contain 0.3 mmol kg^{-1} of InsP3. The highest concentration of InsP4 is present in raw blackeye peas (0.26 mmol kg^{-1}). The InsP5 content in raw dry legumes ranges from 1.36 to 2.52 mmol kg^{-1} in green split peas and blackeye peas, respectively, which is 16% of total inositol phosphates. The most plentiful inositol phosphate in raw dry legumes is InsP6, which ranges from 77% in chickpeas to 88% in black beans and accounts for an average of 83% of the total inositol phosphates (Morris and Hill, 1996; Oomah *et al*., 2008).

16.3.7 Amylase inhibitors

α-amylase inhibitor content varies significantly among legumes, with the highest concentrations present in dry beans. It is present in runner beans and common beans (*Phaseolus coccineus*) in the range 2–4 g kg^{-1}. Blackeyed peas, field beans and chickpeas contain low concentrations in the range 0.1–0.2 g kg^{-1} of seed meal. Its activity is undetected in soybeans, peas, lentils, lima beans (*Phaseolus lunatus*), adzuki beans and winged beans (Grant *et al*., 1995a, 1995b; Genovese and Lajolo, 1998). Based on colour, 150 screened Brazilian bean varieties contain from 0.19 to 0.29 AIUa mg^{-1} of protein α-amylase inhibitor (Table 16.2) with no correlation between seed coat colour and inhibitory activity (Lajolo *et al*., 1991).

16.3.8   Plant phenolics

The polyphenolic compounds of pulses consist mainly of phenolic acids, flavonoids and tannins. Dark and highly pigmented varieties, such as black gram (*Vigna mungo*) and red kidney beans (*Phaseolus vulgaris*), are rich in polyphenolic compounds. Condensed tannins (proanthocyanidins) are quantified in the hulls of numerous varieties of field beans (*Vicia faba*) and also occur in pea seeds of coloured-flower cultivars (Smulikowska *et al.*, 2001).

Pulses vary in their total phenolic contents and antioxidant activities (Table 16.3). Lentils contain the highest quantity of phenolics (6.56 mg gallic acid equivalents g^{-1}), condensed tannin (5.97 mg catechin equivalents g^{-1}) and flavonoid (1.30 mg catechin equivalents g^{-1}), followed by red kidney and black beans. Moreover, lentil, red kidney and black beans with their highest total phenolics exert the highest antioxidant capacity evaluated by 2,2-diphenyl-1-picryhydrazyl (DPPH) free-radical scavenging, ferric-reducing antioxidant power (FRAP) and the oxygen radical absorbance capacity (ORAC) (Xu and Chang, 2007). Ferulic acid is the most plentiful phenolic compound in common beans and intermediate levels of p-coumaric and sinapic acids are also present (Luthria and Pastor-Carrales, 2006). There is a 5-fold variation (3.3–16.6 mg catechin equivalents g^{-1}) in the total phenolic content of six Canadian bean varieties, while variations in anthocyanins, flavonols, flavonoids and tartaric esters are in meagre quantities (Oomah *et al.*, 2005).

Black beans contain chiefly 3-O-glucosides of delphinidin, petunidin and malvidin, while kaempferol and its 3-O-glycosides are present in

Table 16.2   Effect of bean colour on α-amylase inhibitory activity of *P. vulgaris*. An α-amylase inhibitory unit (AIU) value of 10 is defined as a 50% decrease in enzyme activity at 37°C/5 min after addition of 1% starch as substrate. Source: Lajolo and Genovese, 2002. Reproduced with permission of Institute of Food Technologies.

| Bean colour | Specific inhibitory activity (AIUa mg^{-1} of protein) | |
	Range	Average
Light brown	0.09–0.32	0.20
Brown	0.14–0.35	0.29
Dark brown	0.19–0.33	0.25
Pale brown	0.16–0.40	0.29
Beige	0.14–0.40	0.26
Red	0.16–0.37	0.25
Black	0.11–0.30	0.19
White	0.14–0.33	0.23
Purple	0.17–0.22	0.19
Pink	0.16–0.28	0.21

Table 16.3   Phenolic concentrations and antioxidant activities of pulses. Source: Xu and Chang, 2007. Reproduced with permission of Wiley Blackwell.

Legume	Total phenolic content (mg gallic acid equivalents g^{-1})	Total flavonoid content (mg catechin equivalents g^{-1})	Condensed tannin content (mg catechin equivalents g^{-1})	DPPH scavenging capacity (µmol Trolox equivalents g^{-1})	FRAP value (mmol Fe2^{+} equivalents 100 g^{-1})	ORAC value (µmol Trolox equivalents g^{-1})
Chickpea	1.81	0.18	1.05	1.05	0.73	5.13
Yellow pea	1.67	0.18	0.42	2.13	1.28	23.17
Black bean	5.04	2.49	3.40	14.61	9.31	46.22
Green pea	1.53	0.08	0.26	0.91	1.06	3.86
Red kidney	4.98	2.02	3.85	16.92	3.90	24.43
Lentil	6.56	1.30	5.97	16.79	7.78	50.06

pinto beans. Light red kidney beans contain traces of quercetin 3-O-glucoside and its malonates, but pink and dark red kidney beans contain the diglycosides of quercetin and kaempferol. Small red beans have kaempferol 3-O-glucoside and pelargonidin 3-O-glucoside, while flavonoids are invisible in navy, great northern, cranberry and alubia beans (Long-Ze *et al.*, 2008). Further, total anthocyanin in wholegrain and seed coats of 15 cultivars of black beans grown in Mexico falls within the ranges 37.7–71.6 mg g^{-1} grain and 10.1–18.1 mg g^{-1}, respectively. The anthocyanins present in seed coats of beans have been identified as delphinidin 3-glucoside (65.7%), petunidin 3-glucoside (24.3%) and maldivin 3-glucoside (8.7%) (Salinas-Moreno *et al.*, 2005). Chickpeas also contain an ample amount of polyphenolics, such as flavone glycosides, flavonols, oligomeric and polymeric proanthocyanidins which range from 0.92 to 1.68 mg gallic acid equivalents g^{-1} (Zia-Ul-Haq *et al.*, 2008).

In addition, wholegrain cereals are a major source of polyphenols, especially phenolic acids such as ferulic, vanillic, caffeic, syringic, sinapic and p-coumaric acids (Fardet *et al.*, 2008), which have antioxidant properties due to the presence of an aromatic phenolic ring that can stabilize and delocalize the unpaired electron within its aromatic ring (Rice-Evans *et al.*, 1997). They act primarily as free-radical scavengers and/or chelators of trace elements. It is unlikely that their free-radical scavenging capacity is sufficient to explain their antioxidant action *in vivo*, however. Because of their relatively low bioavailability, they range from 0.3 to 26% within the digestive system (Scalbert and Williamson, 2000).

Their mechanism of action may be associated with the activation/repression of particular genes that can act on gene expression via transcription factors (e.g. Nrf2), activating the antioxidant response element (ARE) and leading to the transcription of antioxidant compounds such as glutathione (GSH) or enzymes of glutathione metabolism (Myhrstad *et al.*, 2002; Na and Surh, 2006). In addition, absorbed polyphenols are mostly metabolized and conjugated. For instance, restricted amounts of ferulic and sinapic acids are absorbed which come only from the free and soluble portions that occur in the cereal grains (Kern *et al.*, 2003). Ferulic acid is esterified to arabinose residues in the cell wall arabinoxylans. In the colon, the fermentation process helps to release the bound fractions of ferulic acid and other phenolics (Adom and Liu, 2002). The ferulic acid and its conjugated forms (e.g. glucuronides) that pass the intestinal epithelium apply their antioxidant properties from the aqueous phase of blood plasma, where it is a more effective antioxidant against low-density lipoprotein (LDL) oxidation than the water-soluble antioxidant ascorbic acid (Castelluccio *et al.*, 1996). *In vitro* antioxidant capacities, wheat, oats, barley and rye are considerably allied with their phenolic acid contents which is especially true for ferulic acid, the major phenolic compound in grains except for maize (Adom and Liu, 2002).

Germ and bran fractions are the major contributors to the antioxidant potential which is normally released during the milling process. Coloured varieties of cereals, such as rice, contain more antioxidant capacity than non-coloured varieties (Zieliński and Kozłowska, 2000). The antioxidant potentials of methanolic extracts of cereals are: barley > oat > wheat and rye. Methanolic extracts of red sorghum and black rice have shown higher antioxidant activities than brown rice, white rice, barley, proso and foxtail millets (Choi *et al.*, 2001). Moreover, maize contains the highest *in vitro* antioxidant potential (181 µmol g^{-1} grain) followed by wheat (77 µmol g^{-1} grain), oats (75 µmol g^{-1} grain), and rice (56 µmol g^{-1} grain) (Adom and Liu, 2002). It is suggested that the bound phenolic fractions of two commercial samples of soft and hard wheat have appreciably higher antioxidant capacities than the free and esterified phenolic fractions (Liyana-Patirana and Shahidi, 2006). In fact, the key antioxidant polyphenols are bound polyphenols, which are 58% in barley, 71% in rice, 87% in maize and 90% of the total in wheat (Adom and Liu, 2002).

Cereals such as wheat and rye (>500 µg g⁻¹) also contain significant contents of alkylresorcinols which are phenolic lipids. They have antioxidant properties *in vitro* due to their hydrogen donor and radical scavenging properties (Parikka *et al.*, 2006), but they are less efficient than vitamin E (Kamal-Eldin *et al.*, 2001). Alkylresorcinols are considered as membrane-located antioxidants. They are biologically active and readily absorbed (up to 80%) (Ross *et al.*, 2003) and therefore considered as potential antioxidants *in vivo*. Micromolar concentrations of cereal grain alkylresorcinols can protect erythrocyte membranes against hydrogen-peroxide-induced lipid oxidation (Kozubek and Nienartowicz, 1995).

Some wholegrain cereals are also rich in betaine and choline. Toasted wheat germ contains choline (152 mg 100 g⁻¹ product) and betaine (1240 mg betaine 100 g⁻¹ product); however, the bran fraction contains around 1% betaine. Betaine is a methyl donor to homocysteine, which permits the conversion to methionine. A high concentration of homocysteine is a cardiovascular risk factor and can lead to oxidative stress. Betaine, which is readily available in the digestive tract, may help, together with folates, to reduce oxidative stress *in vivo* by reducing the concentration of homocysteine (Barak *et al.*, 2003; Zeisel *et al.*, 2003). In addition, choline, a precursor of betaine, is also involved in the body's antioxidant defensive mechanism. Wholegrain and refined cereals are also good sources of the sulphur amino acids, methionine and cysteine. Dietary cysteine may involve synthesis of glutathione, a major endogenous antioxidant, predominantly in the liver, and methionine is readily used by the liver to produce cysteine via the cystathionine pathway which also favours glutathione synthesis. Wholegrain and white wheat flours contain 2.3–2.5 g cysteine and 1.2–1.3 g methionine per 100 g protein. Other cereals including barley, oat, rye, rice and maize contain 1.6–2.3 g cysteine and 1.4–2.5 g methionine per 100 g protein (Mariotti *et al.*, 2004; Shewry, 2007).

Rice contains potential antioxidant compounds in the bran of the grain. Significant quantities of vitamin E and γ-oryzanol can be extracted from rice. Since the γ-oryzanol content of rice bran is 10 times that of vitamin E, γ-oryzanol may supply more to the reduction of cholesterol oxidation than vitamin E (Xu *et al.*, 2001). The γ-oryzanol is an assortment of at least 10 phytosteryl ferulates. It is exclusively extracted from rice bran and is a powerful inhibitor of iron-driven hydroxyl radical formation. Some varieties of rice, including coloured varieties, have a greater antioxidant capacity than white rice. Cyanidin-3-glucoside and peonidin 3-glucoside are the key antioxidant compounds in black rice, but they are not found in white rice. Procyanidins are the chief compounds implicated in the antioxidant activity of red rice (Oki *et al.*, 2002; Suh *et al.*, 2005).

The most abundant free phenolic acids in wheat are ferulic, vanillic and p-coumaric acids.

In general, ferulic acid (99–231 µg g⁻¹) is the prime phenolic acid, accounting for 46–67% of total phenolic acids. Like other cereals, corn is a great source of antioxidants, especially ferulic acid, accounting for 2–4% by dry weight of corn. Corn bran may contain up to 26–33 g ferulic acid kg⁻¹ product, which is higher than wheat bran (5.3–5.4 g kg⁻¹), rye bran (2.8 g kg⁻¹), rice endosperm cell wall (9.1 g kg⁻¹) and brown rice (0.4 g kg⁻¹).

Oats also contains large quantities of phytomicronutrients such as vitamin E, phytic acid, phenolic compounds and avenanthramides, which are present in the outer layer of the grain. However, avenanthramide is recognized as a more powerful antioxidant than ferulic acid, vanillic acid, phytic acid and p-hydroxybenzoic acid. Oats contains 8.7 mg kg⁻¹ free phenolic acids, 20.6 mg kg⁻¹ soluble phenolic acids and 57 mg kg⁻¹ insoluble phenolic acids (Hosny and Rosazza, 1997; Peterson *et al.*, 2002; Martinez-Tome *et al.*, 2004; Zhao *et al.*, 2005; Zhou *et al.*, 2005; Liyana-Pathirana and Shahidi, 2006).

Barley is an excellent source of natural antioxidants either for food preservation, or for disease prevention. There is growing interest in barley products due to their high content of

antioxidants including benzoic and cinnamic acid derivatives, quinines, flavonols, proanthocyanidins, chalcones, flavanones, flavones and amino phenolic compounds. Ferulic acid is the most abundant phenolic acid in barley, accounting for 359–624 µg g^{-1} dry weight, as measured in 11 barley varieties (Hernanz *et al.*, 2001; Liu and Yao, 2007).

Rye contains ferulic acid which is the predominant hydroxycinnamic acid and accounts for 900–1170 µg g^{-1} dry matter. The other most plentiful phenolic acids in rye are sinapic acid and the dimer 8-O-4-di ferulic acid which may inhibit LDL oxidation *in vitro*. Sorghum is rich in polyphenols and tannins. The antioxidant potential of sorghum products such as wholegrain, bran and cooked/extruded products is strongly correlated with their polyphenol content. A major proportion (85%) of total phenolic acids is esterified to cell wall compounds. Sorghum bran contains very high antioxidant potential which is about 1000–3000 µmol TE g^{-1} dried product. Purple/red secondary colour sorghum grains, a black or dark-red thick pericarp and a pigmented testa contain greater antioxidant activity. Finger millet is mostly consumed in India, East Africa and Sri Lanka. After malting, it becomes rich in phenolic acids and thus has potential antioxidant activity. The antioxidant capacity of the fraction containing free phenolic acids doubles after 96 h of malting (Andreasen *et al.*, 2001; Subba Rao and Muralikrishna, 2002; Awika *et al.*, 2003; Dykes and Rooney, 2006).

16.3.9   Saponins

In many edible legumes, saponins are reported such as lupins (Woldemichael *et al.*, 2003), lentils (Ruiz *et al.*, 1996) and chickpeas (El-Adawy, 2002), as well as various beans and peas (Shi *et al.*, 2004). Appreciable amounts of saponin are present in chickpeas (3.6 g kg^{-1}), black grams (2.3 g kg^{-1}), moth bean (3.4 g kg^{-1}), broad beans (3.7 g kg^{-1}) and peas (2.5 g kg^{-1}). The saponin content in dehulled light- and dark-coloured peas ranges from 1.2 to 2.3 g kg^{-1} dry matter (Khokhar and Chauhan, 1986; Price *et al.*, 1987; Daveby *et al.*, 1998). Most saponins present as insoluble

complexes with 3-b-hydroxysteroids which interact with bile acid and cholesterol, forming large mixed micelles (Oakenfull and Sidhu, 1989). In addition, they form insoluble saponin–mineral complexes with iron, zinc and calcium (Milgate and Roberts, 1995).

Saponins have long been considered undesirable because of their toxicity and haemolytic activity. However, there is a vast structural multiplicity within this chemical class, and only a few are found toxic. They contain a triterpene or steroid nucleus (the aglycone) with mono- or oligosaccharides attached to this core. The most widespread saponins in legumes are soya saponins, which are categorized as either group A, B and E saponins on the basis of the chemical structure of the aglycone (Rochfort and Panozzo, 2007). Dehydrosoyasaponin-I present in peas has insecticidal and antifeedant characteristics against stored product insect pests. This triterpenoid saponin dehydrosoyasaponin I is a natural product that occurs in chickpeas and other legumes and is known to be a potent calcium-activated potassium channel opener and can be used for treating cardiovascular, respiratory, neurological, urological and other disorders (Taylor and Richards, 2008).

16.3.10   Raffinose

Alpha-galactosides, particularly those of the raffinose family such as stachyose and verbascose (Minorsky, 2003), are plentiful in pulses, ranging from 2 to 10 g 100 g^{-1} dry weight (Guillon and Champ, 2002). Paradoxically, this raffinose family of oligosaccharides (RFOs) are also identified as the primary flatulence-producing compounds in legumes (Granito *et al.*, 2005; Martinez-Villaluenga *et al.*, 2008).

16.3.11   Other antinutrients

Melatonin (N-acetyl-5-methoxytryptamine) has diverse beneficial effects within cells and organisms. This has translated into protective actions against a number of experimental and clinical diseases (Blask *et al.*, 2002; Lissoni, 2002). Melatonin is normally produced in vertebrates, most notably by the pineal gland and

subsequently discharged into the blood and the cerebrospinal fluid (CSF) tool. Given that pineal melatonin is produced in large quantities during the night (or similarly in darkness), blood and CSF melatonin levels are also higher at night than during the day (Reiter, 1991; Lewy, 1999; Skinner and Malpaux, 1999).

The presence of melatonin in some legumes has been reported. The immunoreactive melatonin found in raw lentil seeds (122.7 pg g^{-1}) is lower than that reported in other plants. The concentration of melatonin is enhanced during germination of seeds, achieving the highest values during days 5–7. The increase is found to be 747% in lentil seeds and 620% in vetch seeds after 7 and 6 days of germination, respectively (Zielínski *et al*., 2001). Extensive research has focused on melatonin as a sleep-promoting molecule. Since increased melatonin levels occur at night, coincident with sleep in humans, they are correlated to neurochemical effects and ability to sleep. Moreover, melatonin supplements are also beneficial to those with delayed sleep phase syndrome (Skene *et al*., 1999) or sleep inadequacy that accompanies Alzheimer's disease (Pandi-Perumal *et al*., 2005). As an oncostatic agent it has been found to limit the growth of various tumours, mainly as a suppressor of experimental mammary gland tumours (Molis *et al*., 1995).

The most significant functions of melatonin may relate to its numerous actions in preventing mutilation of essential molecules by free radicals and related reactants (Reiter, 2002).

16.4   Mechanisms of antinutritional factors

There are two distinct mechanisms through which the intrinsic chemicals act as toxicants in the human body. Some naturally occurring chemical compounds exert their harmful effects by binding or destroying particular nutrients present in food, thereby decreasing their availability (Table 16.4). These compounds are dietary fibre, phytates, oxalates and plant phenolics. The dietary fibre reduces the availability of nutrients such as mineral elements, vitamins and proteins, while phytates interfere with the availability of certain trace minerals. Oxalates are known to react with calcium to form a poorly absorbed complex. Some plant phenolics such as tannins bind proteins and convert them into indigestible forms, while others decrease the availability of some mineral elements and vitamins (Veenstra *et al*., 2010).

Another mechanism through which intrinsic chemicals present in plant foods exert their action is by decreasing the absorption or utilization of

Table 16.4 Some intrinsic toxicants of plants that decrease nutrient availability. Source: Awan and Anjum, 2011. Reproduced with permission of the authors.

Toxicant	Nutrients affected	Sources in diet
Dietary fibre	Mineral, vitamins, proteins	Plants
Phytates	Zinc, other minerals	Pulses and cereal grains
Oxalates	Calcium	Spinach, tea, cocoa
Plant phenolics		
Gossypol	Iron, other minerals, proteins	Cottonseed
Tannins	Proteins, vitamin B$_{12}$, glucose	Many plants
Other phenolics	Proteins, vitamins.	Many plants
Vitamin antagonists		
Phenolics	Thiamine	Many plants
Linatine	Pyridoxal	Linseed meal
Enzymes		
Lipoxygenase	Vitamin A	Raw soybeans, other plants
Tocopherol oxidase	Vitamin E	Raw soybeans and kidney beans

Table 16.5   Some intrinsic toxicants of plants that decrease nutrient utilization. Source: Awan and Anjum, 2011. Reproduced with permission of the authors.

Toxicant	Nutrients affected	Mechanism	Source
Enzyme inhibitors			
Trypsin inhibitors	Proteins	Inhibit protein digestion	Legumes
Amylase inhibitors	Carbohydrates	Inhibit carbohydrate digestion	Legumes
Vitamin antagonists			
Linatine	Pyridoxal	Complex formation	Linseed meal
Dicumerol	Vitamin K	Interfere with thrombin production	Sweet clover
Phenolics			
Tannins	Glucose, methionine	Inhibit intestinal absorption	Many plants
Phlorizin	Glucose	Inhibit intestinal absorption	Apples
Saponins	Cholesterol	Inhibit cholesterol absorption	Alfalfa, soybeans
Canavanine	Arginine	Interfere with arginine-dependent processes	*Papilionoidae* plants
Others			
Goitrogens	Iodine	Inhibit iodine uptake by thyroid	*Cruciferae* plants
Nitrite	Iron	Decrease incorporation	Additives, certain plants

one nutrient or the other. Enzyme inhibitors, vitamin antagonists, goitrogens and some plant phenolics fall into this category (Table 16.5). Enzyme inhibitors such as protease and trypsin inhibitors are present in many foods, especially legumes, and can lead to poor utilization of proteins by inhibiting their digestion. However, these compounds are heat-labile and normally destroyed during the cooking process. Some vitamin antagonists (e.g. dicumerol and linatine) act by decreasing the availability of certain vitamins. Similarly, goitrogens interfere with the uptake of iodine by the thyroid, resulting in goitre. Some plant phenolics such as tannins can bind to digestive enzymes, thereby inhibiting their activities (Awan and Anjum, 2010).

16.5   Prevention and detoxification

To prevent hazards associated with toxins naturally present in plant foods, it is important that wild and unfamiliar plants should not be eaten. Consumption of plant foods which have a bitter taste (e.g. bitter almonds) should be avoided.

To prevent an accumulation of toxins in the body, a variety of foods should be eaten. All bean varieties should be well soaked and thoroughly cooked before consumption. Some intrinsic chemical compounds contained in plant foods are drastically reduced or even destroyed when such foods are subjected to certain preparatory or processing operations. Protease inhibitors and haemaglutinins contained in bean varieties such as soybean, black bean, red bean and yellow wax bean are inactivated or destroyed during cooking. There are several methods used for the reduction of plant allergens from peanut, apple, rye grass, wheat, soybean and birch. Legumes are heat treated prior to consumption, particularly for monogastric animals, because they contain toxic compounds such as trypsin inhibitors, amylase inhibitors, lectins, saponins, vicine and convicine accountable for favism in humans. Other compounds such as phytates diminish the availability of iron and phosphate in legumes and, to a lesser extent, in cereals (Farran *et al.*, 2002; Lajolo and Genovese, 2002; Shi *et al.*, 2007). Numerous other treatments such as soaking under different pH conditions and cooking, germination and fermentation

have been developed to reduce the quantity of non-nutritional compounds that impede iron absorption from beans and thereby enhance its bioavailability (Luo *et al.*, 2009).

16.5.1   Soaking in water

Prior to cooking beans, soaking is a common practice to soften their texture. It also reduces the concentrations of antinutritional factors and improves the nutritive value of kidney beans (*Phaseolus vulgaris* L.) (Wah *et al.*, 1997). Soaking cereal and legume grains for 15–20 minutes or longer is useful to facilitate processing. As phytate is water-soluble, a substantial quantity is removed during soaking. Moreover, this process also facilitates the action of naturally occurring phytase in legumes and cereals grains. The phytate hydrolysis is significantly affected by pH and temperature of a substrate during soaking. The optimal temperatures and pH for the naturally occurring plant phytases during soaking are 45–65°C and 5.0–6.0, respectively (Greiner and Konietzny, 1999). However, this process also increases the losses in proteins, vitamins and minerals of beans. Losses in starch content as a result of soaking and cooking are 4.27–24.7% and 30.4–70.7%, respectively. However, starch digestibility of beans is noticeably improved on cooking (Rehman *et al.*, 2001).

Soaking of faba beans (*Vicia faba* L.) leads to a considerable reduction in the quantity of iron (39%), whereas a lower reduction in iron content (10%) is obtained after additional treatment with phytase than in the soaked faba bean flour (Luo and Xie, 2012). Theil (2004) described the good availability of ferritin-associated iron from soybean that results from the ability of this protein to pass largely undigested through the gastrointestinal tract and to cross the mucosal barrier directly into the enterocyte. In contrast, the bioavailability of iron from legumes is usually poor as a result of the presence of non-nutritional components such as phytic acid that may interfere with the absorption of this mineral (Kumar *et al.*, 2010). The bioavailability of iron, phosphorus and other essential minerals from bean-supplemented diets

can be significantly increased by dephytinization or by the direct addition of phytase in the diet (Luo *et al.*, 2010).

16.5.2   Boiling/steeping/steaming

Investigations into the effects of soaking, boiling and steaming processes on the total phenolic components and antioxidant activity in commonly consumed cool-season legumes (CSLs) such as yellow pea, green pea, lentil and chickpea have been conducted. Significant decrease in total phenolic content (TPC) and DPPH free-radical scavenging activity has been observed in all processing treatments. However, all soaking and atmospheric boiling treatments decrease the oxygen radical absorbing capacity (ORAC) while pressure boiling and steaming increase the ORAC. Steaming results in a greater retention of TPC, DPPH and ORAC values than boiling treatments. However, TPC and DPPH in cooked lentils differ considerably between atmospheric and pressure boiling. Pressure processes appreciably increased ORAC values in both boiled and steamed CSLs than atmospheric processes. Greater TPC, DPPH and ORAC values were detected in boiling water than in soaking and steaming water. Boiling also causes more solid loss than steaming (Xu and Chang, 2008). Jahan and Ahmad (1984) described the detoxification of vetch (*Lathyrus sativus* L.) by steeping followed by boiling in water. Lime water and autoclave treatments of seeds for 10 minutes at 15 lb mm^{-2} destroyed the toxin and trypsin inhibitors. In another study, whole seeds were boiled for 2 h and dehusked. After one hour, the cotyledons had lost about 53% of soluble protein, while losses from whole seeds were about 47%. However, toxic amino acids were reduced by 79% in the cotyledons and 78% in whole seeds after two hours (Jha 1987). Vetch (*Lathyrus sativus* L.) is detoxified to the extent of 97% by steeping in double its quantity of water for 8 h at 70°C, changing the water seven times, draining and sun drying (Rehman *et al.*, 2006a). This can be partially supplemented for the preparation of

food products such as matri-skim milk-blend ice cream (Rehman *et al.*, 2004), pizza cheese (Rehman *et al.*, 2008), chapatti (Rehman *et al.*, 2006b), doughnuts (Rehman *et al.*, 2007) and naan (Farooq *et al.*, 2012).

16.5.3   Germination and malting

Germination and cooking improves the nutritional quality and protein digestibility of beans (Rehman and Shah, 1996). Phytate is a heat-stable component in plant food which is not easily degraded by cooking. However, the natural plant phytase is thermo-labile and extended exposure to high temperature may lead to its inactivation. To improve phytate dephosphorylation during cooking, plants with heat-stable phytases or addition of exogenous heat-stable phytases are recommended. During the germination of cereals and legumes, phytate is degraded by native phytase. Plant seeds utilize phytate as a source of inorganic phosphate during germination and hence tend to improve flavour and nutritional value. Several investigators have reported slight intrinsic phytate-degrading activity in non-germinated legume and cereal grains with the exception of wheat, barley, rye and triticale (Viveros *et al.*, 2000; Egli *et al.*, 2002). During germination of cereals and legumes, an increase in phytate-degrading activity, with a concomitant decline in phytate content, has been observed (Greiner *et al.*, 2001). Furthermore, when malted cereals (except for oats) are ground and soaked under optimal conditions, a complete degradation of phytate is observed (Larsson and Sandberg, 1992).

16.5.4   Fermentation

Fermentation is applied to extend the shelf life of food by using microbes and enzymes. Lactic fermentation is ideal for the fermentation of cereals and legumes. Bacterial production of lactic and acetic acids lowers pH which exerts a positive effect on phytase activities, lowering phytate (Lopez *et al.*, 2002). The acidity of the dough plays a vital role in phytate degradation during scalding and sourdough fermentation of bread

(Rehman *et al.*, 2006a; Rehman and Awan, 2011). In case of oat and rye bran bread made with 10% sourdough of pH 4.6, a 96–97% reduction in phytate content is occurred (Larsson and Sandberg, 1991). Moreover, combined germination and lactic fermentation of white sorghum and maize gruels may lead to complete degradation of phytate (Svanberg *et al.*, 1993). Food products such as miso, kiji, tempeh and soy sauce are produced by fermentation of soybeans with *Aspergillus oryzae* and *Rhizopus oligosporus* (Fujita *et al.*, 2003). Lectin may be entirely depleted of lentil flour by fermentation for 72 h at 42°C with a flour concentration of 79 g L^{-1} (Cuadradol *et al.*, 2002).

16.6   Health repercussions

Legumes play an important role in lowering serum cholesterol and increasing the saturation levels of cholesterol in the bile. A dietary study conducted on humans for seven weeks showed that serum LDL cholesterol significantly reduced during the consumption of a diet containing beans, lentils and field peas. These have the effect of lowering LDL cholesterol by partially interrupting the entrohepatic circulation of the bile acids and increasing the cholesterol saturation by increasing the hepatic secretion of cholesterol (Lajolo and Genovese, 2002). The addition of a black bean inhibitor in a starch meal has been shown to slow starch digestion in stomach with reduction of serum glucose and insulin concentrations and increased metabolism of non-esterified fatty acids from the adipose tissue in rats. Correspondingly, in a 10-day experiment with purified α-amylase inhibitor, reduced utilization of dietary starch and protein for rats has been observed (Lajolo *et al.*, 1991; Pusztai *et al.*, 1995). Lectin is among the major protein found in lentils (*Lens culinaris*). All lectins fasten one transition ion, typically manganese, and one calcium ion. Intact and decorticated lentils demonstrate both manganese ion and radical signals; however, the testa exhibits only the radical signal (Polat and Kormaz, 2001). Lectins are also employed

for the discovery of protein markers of cancer by using a natural glycoprotein microarray approach.

Multiple lectins may screen serum samples from patients with pancreatic cancer or pancreatitis by selective detection of glycan structures. Lectin from kidney bean seeds directly inhibits HIV-1 reverse transcriptase, an enzyme crucial for HIV replication as well as β-glucosidase, which has a role in processing HIV-1 envelope protein gp120 and is therefore a very potent element of the antiretroviral chemotherapy (Zhang *et al.*, 2009).

Several cereal and legume lectins may inhibit the growth of experimental animals due to reduction in the digestibility and biological value of dietary proteins. They may impair the integrity of the intestinal epithelium and so alter the absorption and utilization of nutrients. Dietary lectins are therefore usually considered to be toxic and antinutritional factors. However, many lectins are non-toxic, such as those extracted from lentils, peas, faba beans, chickpeas, tomatoes and other foods (Grant *et al.*, 2000; Radberg *et al.*, 2001; Reynoso-Camacho *et al.*, 2003).

Many studies have revealed a strong association between certain lectin-binding patterns and their biological behaviour in various tumours. Vicia faba agglutinin (VFA), a lectin present in broad beans, stimulates the morphological differentiation and reduces the malignant phenotype of colon cancer cells (Jordinson *et al.*, 1999). The addition of phytohemagglutinin (PHA) in the diet, a lectin present in raw kidney bean (*Phaseolus vulgaris*), significantly reduces the growth of a murine non-Hodgkin lymphoma tumour in the mouse, either as an intraperitoneal ascites tumour or as a solid subcutaneous tumour (Pryme and Bardocz, 2001).

Besides having harmful, phytate intake provides protection against several types of cancers mediated through antioxidation properties, interruption of cellular signal transduction, cell cycle inhibition and enhancement of natural killer (NK) cells activity. It also has curative use against atherosclerosis, diabetes mellitus, kidney stone formation, HIV-1, heavy metal toxicity and coronary heart disease (Kumar *et al.*, 2010). Phytic acid delays postprandial glucose absorption, reduces the bioavailability of toxic heavy metals such as lead and cadmium and exhibits antioxidant activity by chelating copper and iron (Minihane and Rimbach, 2002). Dietary and endogenous phytic acid has defensive effects against heart disease and may be responsible for the cancer-protective effects of high-fibre foods (Fredlund *et al.*, 2006). It may reduce the incidence of colonic cancer by a similar mechanism and protect against other inflammatory bowel diseases (Graf and Eaton, 1990). Moreover, it inhibits xanthine oxidase-induced superoxide-dependent DNA damage (Muraoka and Miura, 2004).

Xanthine oxidase, which generates superoxide anions (O_2^-) during the oxidation of xanthine, is abundant within the intestine (Battelli *et al.*, 1972). *In vitro* and *in vivo* studies have confirmed that inositol hexaphosphate (InsP6, phytic acid) shows significant anticancer properties. It decreases cell proliferation and enhances differentiation of malignant cells with possible reversion to the normal phenotype. It is involved in host defence mechanism and tumour abrogation (Shamsuddin, 2002). The backbone of most inositol phosphates in cells is myo-inositol. Inositol phosphates from grains are a major food source of myo-inositol, as are the phospholipids and free inositol from many plant- and animal-based foods (Berdanier, 1992). In mice, dietary myo-inositol has been demonstrated to be effective in preventing cancer of the lung (Wattenberg *et al.*, 2000), liver (Nishino *et al.*, 1999), fore-stomach (Estensen and Wattenberg, 1993), mammary glands, colon, prostate and skin (Jenab and Thompson, 1998, 2002).

The majority of plants of the Fabaceae/Leguminosae family contain an appreciable amount of flavones and isoflavones. Chickpeas contain daidzein (0.04 mg 100 g^{-1}), genistein (0.06 mg 100 g^{-1}), formononetin (0.14 mg 100 g^{-1}) and around 1.7 mg 100 g^{-1} biochanin A. Soybeans contain significantly higher levels of daidzein (47 mg 100 g^{-1}) and genistein (74 mg 100 g^{-1}) but have less formononetin (0.03 mg 100 g^{-1}) and biochanin A (0.07 mg 100 g^{-1}) compared to chickpeas.

There are several biological activities related to isoflavones such as reduction in osteoporosis, cardiovascular disease, prevention of cancer and treatment of menopause symptoms (Cassidy *et al.*, 2006; Trock *et al.*, 2006). Barceló and Muñoz (1989) identified isoflavones such as genistein, 2′ hydroxigenistein, luteone and wighteone in sprouted hypocotyls of *L. albus CV multolupa*, describing how these are related to the lignification of the cell wall. The cotyledon of Andean lupins contains a higher quantity of total isoflavones (16–31 mg 100 g^{-1} cotyledon FW) compared to the hypocotyls (1.3–6.1 mg 100 g^{-1} hypocotyls FW) and seed coats (9.8–10 mg 100 g−1 seed coat FW).

Epidemiological studies have demonstrated that legume saponins may possess anti-cancerous activity (Chang *et al.*, 2006) and beneficial for hyperlipidemia (Shi *et al.*, 2004). Moreover, the consumption of these compounds through a diet rich in food legumes reduces the danger of heart diseases in humans (Geil and Anderson, 1994). Metastatic actions are dangerous in cancer propagation and glycosylation is a key event in this process, demonstrating that soyasaponin I decreases the expression of R-2, 3-linked sialic acid on the cell surface and in turn restrains the metastatic potential of melanoma cells (Chang *et al.*, 2006). There is also evidence for saponin regulation of the apoptosis pathway enzymes (AKT, Bcl and ERK1/2) which accompanies the programmed cell death of cancer cells (Xiao *et al.*, 2007).

Soluble dietary fibre can reduce total and low-density lipoprotein, cholesterol levels and insulin resistance (Glore *et al.*, 1994). Consumption of legumes has been linked to reduced risk of coronary heart disease and cardiovascular disease (CVD); consumption of legumes four times or more per week compared to less than once a week may be associated with 22% lower risk of CHD and 11% lower risk of CVD (Flight and Clifton, 2006). The effects of azuki bean juice upplementation on serum lipid concentrations, prescribed according to a Kanpo medicine regimen, were studied in young Japanese women. Triglyceride concentrations decreased in the azuki juice group, mediated by inhibited pancreatic lipase activity. Azuki juice intake might be beneficial in preventing hypertriglyceridemia (Maruyama *et al.*, 2008).

Mung bean (*Vigna radiata*) is an excellent source of protein with its essential amino acid profile comparable to that of soybean and kidney bean (Mubarak, 2005). Its consumption produces a small increase in the blood glycemic index in humans, making it a striking option for diabetic patients. Moreover, certain proteins in mung bean have demonstrated antibacterial and anti-fungal activities (Wang *et al.*, 2004). The key feature of the metabolic syndrome is obesity, which may be controlled by consuming a high quantity of wholegrain foods such as cereals and legumes.

However, apprehension has been voiced that increased intake of refined grains may lead to an increase in obesity (Koh-Banerjee and Rimm, 2003). Chickpeas are the major legume crop grown in Pakistan, and several varieties are commonly consumed as a source of dietary protein. Seeds enrich the blood and cure skin diseases and inflammation of the ear (Agharkar, 1991). They are used as an appetizer, tonic, stimulant and aphrodisiac and have anthelmintic properties (Sastry and Kavathekar, 1990). Dietary supplementation with chickpeas results in considerable reduction in total serum and low-density lipoprotein cholesterols in adult woman and men (Table 16.6). (Pittaway *et al.*, 2006).

16.7   Conclusions and future outlook

Plant-derived toxins and allergens are abundantly present and fundamentally unavoidable compounds of our diet. Legumes and cereals contain numerous deleterious compounds such as enzyme inhibitors, lectins, phytates, oligosaccharides, and phenolic compounds. Enzyme inhibitors may reduce protein digestibility, while lectins may reduce nutrient absorption. Phytic acid reduces the bioavailability of minerals. Some phenolic compounds diminish protein digestibility and bioavailability of minerals, and galactooligosaccharides cause flatulence. However, these compounds may

Table 16.6   Beneficial effects of legumes.

Source	Involved metabolism	Beneficial effect	Reference
Legumes (including some pulses)	Cardiovascular	22% lower risk of coronary heart disease, 11% lower risk of cardiovascular disease	Bazzano *et al.* (2001)
	Cardiovascular and diabetes	Modulation of glucose, insulin and homocysteine concentrations and lipid peroxidation in coronary artery disease patients	Jang *et al.* (2001)
Legumes and cereals	Obesity	Low average body mass index (BMI) and low risk of obesity	Greenwood *et al.* (2000)
Mung bean	Glucose and lipid metabolism	Modify glucose and lipid metabolism favourably in rats	Lerer-Metzger *et al.* (1996)
Legumes	Type II diabetes mellitus	Risk reduction of developing T2DM of the order 20–30%	Venn and Mann (2004)
Azuki bean juice	Hypertriglyceridemia	Decreased triglyceride concentrations by inhibited pancreatic lipase activity	Maruyama *et al.* (2008)
Legumes	Endometrial cancer	Low risk of endometrial cancer	Tao *et al.* (2005)
	Breast cancer	Low breast cancer risk	Velie *et al.* (2005)
	Colon cancer	Low risk of colorectal adenoma	Agurs-Collins *et al.* (2006)
Wholegrain bread and beans	Glycaemia and obesity	Glycaemic control and weight loss	Jimenez-Cruz *et al.* (2003)
Vegetables (including some seeds of pulses)	Lymphoblastic leukaemia	Low risk of lymphoblastic leukaemia	Petridou *et al.* (2005)
Wholegrains, beans and legumes	Obesity	Low body mass index and waist circumference (WC)	Haveman-Nies *et al.* (2001)
Pulses	Obesity	Low waist-to-hip (WHR) ratio	Williams *et al.* (2000)
Chickpeas	Skin and ear inflammation	Low risk of skin diseases and inflammation of the ear	Agharkar (1991); Warrier *et al.* (1995)
		Tonic, appetizer, stimulant and aphrodisiac, anthelmintic properties	Sastry and Kavthekar (1990)
	Hypertriglyceridemia	Reductions in serum total and low-density lipoprotein cholesterols	Pittaway *et al.* (2006)

have protective and health benefits. Phytic acid has antioxidant properties and protects against DNA damage while phenolic compounds have antioxidant as well as other important physiological and biological properties. The Galactooligosaccharides may encourage prebiotic activity in the human body. Moreover, these compounds play a key role in stimulation of the immune system, detoxifying enzymes, regulation of lipid and hormone metabolism, have antimutagen, antioxidant and antiangiogenic effects, and can reduce the incidence of colon cancer and tumor initiation. Lectin from kidney bean seeds is known to inhibit HIV-1 reverse transcriptase, an enzyme crucial for HIV replication.

The human body has developed built-in mechanisms through which most intrinsic or other toxic substances are either converted into harmless compounds or excreted from the body. However, plant-derived food sources must be heat-treated before consumption in order to diminish their toxic factors. These have little influence after cooking.

Considering the health benefits of secondary metabolites, these may be marketed as functional and nutraceutical ingredients. Future research should be focused on isolation, characterization, immunological responses and their heath benefit issues.

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17
Nanotechnology Tools to Achieve Food Safety

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Summary

Food safety has been of major concern since ancient times, and several technological approaches have been proposed to ensure this. Nanotechnology is among the emerging technologies available to achieve this goal today. Nanotechnology has been used for developing food safety, including: rapid localization and removal of contaminated lots from the market; microbial and chemical risk sensors to detect contaminated food; and controlled release of antimicrobial agents. The latter is an area that has developed considerably and can be useful to reduce biological risks. In the present chapter some of these applications are discussed, paying special attention to safety regulations applied to nanotechnology-derived materials and their possible interaction with food constituents.

17.1 Introduction

There is much interest worldwide in food safety as a result of well-publicized outbreaks due to foodborne pathogens. The four food categories (excluding multi-ingredient foods) linked to the most foodborne illness outbreaks were seafood, produce, poultry, and beef. These four categories were responsible for 53% of all outbreaks and

48% of all illnesses. Although seafood had the highest number of outbreaks after the multi-ingredient category, the average outbreak size was small (CSPI, 2013). The increase in diseases associated with fruits and vegetables in 2008 may have been due to increased consumption of uncooked vegetables in salad, sandwiches, at ready-to-eat food bars, ethnic foods and others, and to the increase in fresh-cut or minimal processing of vegetables and fruits. An annual list relevant to the outbreaks during 2007–2013 was compiled by the Center for Science in the Public Interest. With food safety and pathogens, the path forward is clear: increase monitoring and enforcement, develop and apply good hygiene and disinfection, and communicate risks broadly to the consuming public.

Although nanotechnology applications for the food sector are relatively recent, there have been rapid developments in this area in recent years, especially in the food safety assurance area. The main developments so far have been aimed at encapsulating and controlling the release of antimicrobial additives and pathogen risk sensors (Duncan, 2011; Lloret *et al.*, 2012). The currently known and projected applications of nanotechnology for food safety are broadly discussed in this chapter: nanosized, nanoencapsulated or engineered nanoparticles as antimicrobial additives used in food; nanomaterials incorporated as 'active' or 'intelligent' materials for food packaging; and nanosensors for food safety and traceability.

17.2   Types of nanotechnological devices

Antimicrobial nanosystems can be divided in two groups according to their mechanism of action: where the antimicrobial is released from nanocapsules to the headspace of the package in order to interact with the product surface (Figure 17.1); and where the antimicrobial compound is immobilized in the surface of the package using nanocomposite materials (Figure 17.2).
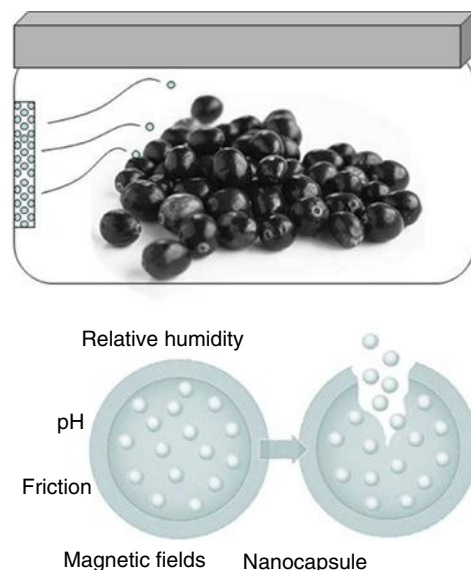
Figure 17.1   Controlled release nanosystems of antimicrobial compounds triggered using different stimuli and incorporated in-package of food matrices to offer protection throughout the shelf-life.
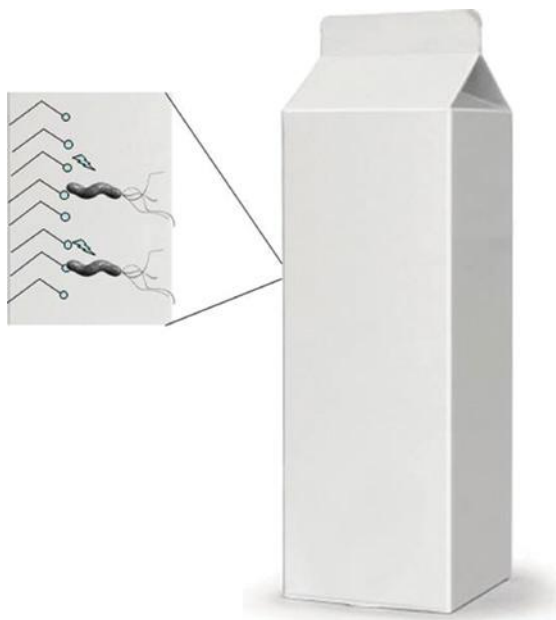
Figure 17.2   In-package immobilized antimicrobial agents using nanoassemblies; direct contact among pathogens and nanosystem is required for action.

17.2.1 Nanosystems to release antimicrobial compounds

These systems are used to design active packaging in the form of sachets or active plastic films containing the nanocapsules that are enclosed in the interior of the package. They can be divided into two groups: indirect and direct antimicrobial activity. Nanosystems with indirect antimicrobial activity include oxygen and moisture scavengers and carbon dioxide absorbing/emitters. They are considered indirect antimicrobial agents because, even although their primary activity is to decrease spoilage due to enzymatic deteriorative reactions and alter the internal atmosphere (decrease of oxygen and moisture), they inhibit the growth of aerobic bacteria. Headspace artifacts with direct antimicrobial activity include antimicrobial volatile compounds such as essential oils (Ayala-Zavala *et al.* 2008b; Azam *et al.* 2012; Espitia *et al.* 2012). A cyclodextrin-essential oil nanocapsule that was used as a headspace nanosystem to increase the shelf-life of fresh-cut produce was described by Ayala-Zavala and Gonzalez-Aguilar (2010). In this study it was hypothesized that internal moisture can be the driving force that releases the antimicrobial compound from the molecular complex.

Another type of antimicrobial active packaging artifact is that in which the antimicrobial compound is embedded in the bulk polymer within nanocavities and has to migrate to the surface in order to interact with the microorganism. Different natural and synthetic polymers have been used as carriers; several reviews on this subject have been recently published (Silvestre *et al.*, 2011; Sonawane *et al.*, 2011). Mono- and multi-layer antimicrobial packages using this technology have been developed (Duncan, 2011). Typical multilayer films consist of four layers: outer layer, barrier layer, matrix layer (in which the antimicrobial is embedded), and control layer. Several organic and inorganic compounds have been used as antimicrobials, including silver zeolites, organic acids and their derivatives, peptides, enzymes, essential oils parabens, bacterocins, and volatile compounds, among others (Arana-Sanchez *et al.*, 2010; Imran *et al.*, 2010; Duncan, 2011; Llorens *et al.*, 2012; Seil and Webster, 2012). One of the main disadvantages of this kind of package is that heat-sensitive compounds cannot be used because they are inactivated during the processing of the package. An interesting option is the use of nanoencapsulation of active compounds in the case of heat-sensitive antimicrobials before incorporation into the polymer extrusion process (Hatzigrigoriou and Papaspyrides, 2011).

Controlled release of the active compound into the headspace of the package is regulated by several factors that include heat, pH, relative humidity (RH), enzymatic activity, and physical modifications of the host, guest, or package, among others (Figure 17.1) (Ho *et al.*, 2011). The effect of these factors is due to chemical interactions between both host and guest such as hydrogen bonding, Van der Walls interactions, and other non-covalent interactions which depend mainly on the polarity, molecular weight, polydispersity and cross-linking of the host molecule (Madene *et al.*, 2006; Duncan, 2011) as well as its ability to undergo a reversible phase transition (Augustin and Hemar, 2008). Among these factors, relative humidity seems to be the most important in the release of antimicrobial compounds (Ayala-Zavala *et al.* 2008a; Mastromatteo *et al.* 2010; Ho *et al.* 2011).

Controlled release of antimicrobial compounds (volatiles) into the headspace can be analyzed considering a zero-order or first-order kinetic model. There are two mathematical models used to describe these kinetic processes. The first is the Power Law (Equation (17.1)) and the second is Avrami's equation (Equation (17.2)), defined:

$$X = kt^{n_P} \tag{17.1}$$

$$X = 1 - e^{-kt^{n_A}} \tag{17.2}$$

where X is the release fraction of nanoencapsulated antimicrobial compound at time t; k is the release rate constant; n_P is the diffusive release parameter; and n_A is the Avrami parameter or release mechanism. In both models, when $n \sim 0.5$ the active agent is released by a Fickian diffusion

mechanism. However, when $n \sim 1$, it describes a zero-order release model using the Power Law model (release independent of concentration) or first-order release model when analyzed by the Avrami's model. Recently Ho *et al.* (2011) reported that at low RH both models were able to describe the release of ethylene from beta-cyclodextrin; however, at high RH (93%), Avrami's model better described the system. These authors reported a 20-fold release of ethylene at this RH compare to 53% humidity. Similar results were observed for essential oils (Ayala-Zavala *et al.*, 2008a) and isothiocyanate encapsulated in cyclodextrins (Li *et al.*, 2007) and D-limonene encapsulated in carbohydrate carriers (Soottitantawat *et al.*, 2004).

When the antimicrobial compound is released from the encapsulating material to the food by direct contact, the release mechanism is explained by considering a migration process which follows a Fick's Law diffusion process. The release of an active ingredient from the packing material is regulated by three different mechanisms: (1) diffusion of the active ingredient through the polymeric material of the packaging; (2) erosion of the polymeric material causing the dispersion of the active ingredient into the food; and (3) swelling or hydration of the polymeric material (Pothakamury and Barbosa-Cánovas, 1995; Mastromatteo *et al.*, 2010; Fathi *et al.*, 2012).

The most commonly used equation for the analysis of the controlled release through a diffusion process is the Huguchi equation (Equation (17.3)) which describes a square root of time-release kinetics (Siepmann and Peppas, 2011; Fathi *et al.*, 2012):

$$X = \sqrt{DC_m(2C_i - C_s)t} \qquad (17.3)$$

where D is the diffusion coefficient of the active ingredient; C_m is the solubility of the active ingredient in the encapsulant matrix; C_i is the initial active ingredient concentration; and C_s is solubility of the active ingredient in the exterior. The limiting condition necessary to use this equation is that a pseudo-steady-state is achieved, which is only obtained when the initial concentration is

much higher than the exterior concentration. The use and misuse of this equation has been recently reviewed by Siepmann and Peppas (2011). For hydrophilic active ingredients where swelling and erosion plays an important role, the Power Law model (Equation (17.1)) is used to describe the release of antimicrobial compounds into the food matrixes (Mastromatteo *et al.*, 2010; Fathi *et al.*, 2012).

17.2.2   Immobilization of antimicrobial compounds using nanocomposite materials

There are few examples of antimicrobial packages in which the antimicrobial compound has been immobilized with nanoassemblies into the polymer by ionic or covalent bonds. In order to attach the antimicrobial, the presence of functional groups in both the polymer and antimicrobial is necessary (Figure 17.2). The presence of a flexible linking group is also desirable, in order to give more flexibility to the antimicrobial and consequently increase its antimicrobial effect (Jin *et al.*, 2008; Duncan, 2011). Extensive research has been conducted into the design of antimicrobial immobilized packages without any specific application to fresh produce.

Silver zeolite is the most-used immobilized antimicrobial; Busolo *et al.* (2010) reported that a silver-based nanoclay composite reduced the number of *Salmonella* spp. colonies by up to eight orders of magnitude with regard to the control. The antimicrobial activity of silver composite has been associated with several actions, including: adhesion and rupture of cell surface; degradation of lipopolysacharides; increase in permeability; and binding of silver to electron donor groups in biological molecules containing sulfur, oxygen, and nitrogen (Azeredo, 2009).

Titanium dioxide has been used as a photocatalytic disinfectant for surface coating, due to its ability to promote peroxidation of polyunsaturated phospholipids of microbial cell membranes (Azeredo, 2009). Chawengkijwanich and Hayata (2008) developed a TiO_2 powder-coated

packaging film able to reduce *E. coli* in fresh-cut produce.

Emamifar *et al.* (2010) observed that a ZnO-low-density polyethylene nanocomposite reduced the microbiological spoilage of orange juice compared to the control, without impairing its sensory properties. Other immobilized antimicrobials include peptides, enzymes, polyamines and organic acids (de Abreu *et al.*, 2012).

Nanoscale chitosan has been reported to demonstrate antimicrobial activity due to the electrostatic interactions between positively charged chitosan molecules and negatively charged cell membrane molecules, increasing membrane permeability. However, due to its possible cytotoxicity, incorporation of chitosan in food packaging materials is still not recommended (Azeredo, 2009).

Several antimicrobial peptides have been immobilized in surface-modified polystyrene showing good microcidal activity in a time- and concentration-dependent manner against several bacteria, molds, and yeast (Appendini and Hotchkiss, 2002). The main inconvenience of this kind of active packaging is that, in order to inhibit the microorganism growth, direct contact between the fresh produce and the polymer is necessary. In this context, the design of wrapping films could be desirable to ensure the beneficial effect of maintaining the quality of food.

Another interesting application of immobilized antimicrobial compounds is the development of modified cellulose-silver nanocomposite absorbent pads. Fernandez *et al.* (2010) observed a 3-log reduction of microflora compared to the control of fresh-cut melon pieces stored under a modified atmosphere when cellulose-silver absorbent pads were used.

17.3   Food safety monitoring systems

The development of new or improved analytical methods that can be used in the prevention of disease, source tracking, and determining the cause of foodborne disease is an expanding area of research. Nanotechnology has benefited the area of food safety mostly through the development of highly sensitive and low-cost nanosensors. They can be incorporated into food packaging matrices and have the ability to identify specific microbial and/or chemical contaminants or environmental conditions. These can respond in a way that alerts the consumer to the contamination (Neethirajan and Jayas, 2011). Nanosensors can provide quality assurance by tracking microbes, toxins and contaminants throughout processing. This is not only useful for quality control to ensure that consumers are able to purchase products which are at their peak of freshness and flavor, but also has the potential to improve food safety and reduce the frequency of foodborne illnesses.

Nanotechnology-based sensors have the potential to revolutionize the speed and accuracy with which industries or regulatory agencies can detect the presence of molecular contaminants or adulterants in complex food matrices (Lachance, 2004). Nanotechnology offers many technological advances for pathogen and toxin detection. The use of nanoparticles as labels in conjunction with novel detection technologies has led to improvements in sensitivity and multiplexing capabilities. Some of the most commonly used nanosensors for the detection of microbial growth and toxins in the food sector are described in the following sections.

17.3.1   Microbial growth nanosensors

The ability to determine whether food products are contaminated by various bacteria, fungi, viruses, or toxins that can cause foodborne illnesses remains an important research objective. Conventional molecular diagnostic techniques are widely used in laboratories throughout the world to identify pathogenic agents with a high degree of sensitivity and reproducibility. However, most of these techniques cannot be utilized in the field (e.g. food distribution centers, food packaging). Hence, taking advantage of the unique electrical, magnetic, luminescent, and catalytic properties of nanomaterials, pathogen detection strategies are increasingly abandoning conventional microbiological analysis methods

in preference of a reliance on nanomaterials themselves as the means of detection (Merkoci, 2010). In this sense, faster, sensitive, and more economical diagnostic assays are being developed to assist in the battle against microbial growth. Apart from striving for sensitivity and speed, nanotechnologists have geared their efforts towards the development of nanotechnology-based systems that are affordable, robust, and reproducible, making them suitable for applications.

Among the near-market developments are nanomaterial-based next-generation packaging displays that include a radio-frequency identification display (RFID). These displays utilize smart labels that will assist quick and accurate distribution of a wide variety of goods with limited shelf-life (Figure 17.3). The RFID systems will be designed to operate automatically and will provide exception reports for anomalies such as temperature and short-lifespan products (Silvestre *et al.* 2011). Metallic nanoparticles composed of gold or silver have many optical and electronic properties, derived from their size and composition (Nath *et al.*, 2009). When coupled with affinity ligands, these nanomaterials find important applications as chemical sensors. For example, gold nanoparticles conjugated with specific oligonucleotides can sense complementary DNA strands, detectable by color changes (Figure 17.4) (Mirkin *et al.*, 1996; Elghanian *et al.* 1997).

Fluorescent quantum dots, fluorescent silica nanoparticles and carbon nanotubes have been used in various applications including DNA detection and the development of immunoassays for the detection of bacteria and toxins (Goldman *et al.*, 2004; Edgar *et al.*, 2006). For bacterial detection, a method for possible quantitation of bacteria based on the emission wavelength shifts of quantum dots (Qdots) on binding with bacteria has been reported by Dwarakanath *et al.* (2004). The test was done for *Salmonella* Typhimurium, *Escherichia coli* and *Bacillus subtilis* spores using CdSe/ZnS Qdots. Both antibody-Qdot and DNA aptamer-Qdot conjugates exhibited a dramatic blue shift of at least 140 nm upon binding to the bacterial surface. The authors attributed this shift to the changes in the chemical environment and physical deformation of the Qdot conjugate upon binding to bacteria. Zhao *et al.* (2009) reported a method for simultaneous detection of foodborne pathogenic *E. coli* O157:H7, *S.* Typhimurium and *Shigella flexneri* bacteria.

(a)

(b)
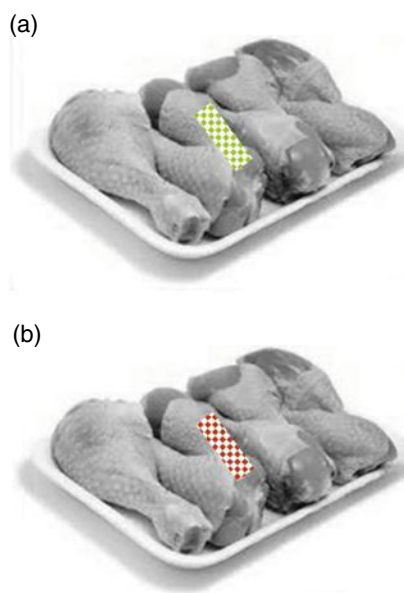
Figure 17.4 Colorimetric nanosensor for bacterial growth. When the produce is fresh the label color is (a) green; when the product is contaminated the label color (b) changes to red, communicating risk to consumers or retailers. For color details, see color plates section.

Figure 17.3 Bacterial sensor nanosystem activated by the pathogen growth on food surfaces to communicate risk.

Horner *et al.* (2006) developed an analytical technology called reflective interferometry, using nanotechnology which provides specific, rapid, and label-free optical detection of biomolecules in complex mixtures. This new platform technology has provided food quality assurance by detecting *E. coli* bacteria in a food sample by measuring and detecting light scattering by cell mitochondria. This nanosensor works on the principle that a protein of a known and characterized bacterium set on a silicon chip can bind with any other *E. coli* bacteria present in the food sample. This binding will result in a nanosized light scattering, detectable by analysis of digital images.

A biosensor developed by Fu *et al.* (2008) uses fluorescent dye particles attached to anti-salmonella antibodies on a silicon/gold nanorod array. When the *Salmonella* bacteria present in the food is being tested, the nanosized dye particles on the sensor become visible.

A significant problem with sensing in complex matrices such as foods is dealing with sample opacity, light scattering, color, and other numerous interferences. Although some all-optical methods have been devised, most detection strategies in real food systems require isolation of the target organism from the surrounding environment to ensure that signal-to-noise ratios are sufficiently large to observe. Often, a technique known as immunomagnetic separation (IMS) is used to satisfy this requirement (Fluit *et al.*, 1993). IMS uses magnetic particles attached to selective antibodies in combination with a magnet to selectively separate the target analyte from the food matrix prior to detection. Electrochemical detection of microorganisms is also a popular and efficient method when it comes to nanomaterials.

An example of this strategy is conductive TiO_2 nanowire bundles coated with antibodies selective for *Listeria monocytogenes* and deposited between two gold electrodes (Wang *et al.*, 2009). In contaminated samples, bacteria bind to the antibodies which causes a measurable change in impedance across the nanowire bundle. Using this technique, the authors were able to detect *L. monocytogenes* in 1 hour without significant interference from other foodborne pathogens.

The development of nanosensors that serve as an 'electronic nose' for pathogen detection has received considerable attention in recent years. Such nanosensors could be placed directly into the packaging material, where they would serve as an 'electronic tongue' or 'nose' by detecting chemicals released during microbial growth in food (Lange *et al.*, 2002). Conducting polymers have been used as detectors in electronic nose nanosystems. When gas is adsorbed by the nanosensor, conducting organic polymer sensors exhibit a change in resistance which is sensed and delivered as the output (Arshak *et al.*, 2009).

Conducting-polymer-based e-nose sensors for foodborne pathogen detection has been reported by many researchers. Magan *et al.* (2001) used Bloodhound ™ BH114, an electronic nose unit including 14 conducting polymer nanosensors, to detect the volatile profiles produced by uninoculated skimmed milk media or that inoculated with bacteria (*Pseudomonas aureofaciens*, *P. fluorescens*, and *B. cereus*) or yeasts (Figure 17.2). It has been reported that, by using discriminant function analyses, it was possible to separate unspoiled milk and milk containing spoilage bacteria or yeasts.

Nanobiosensors based on electronic conducting polymers were reported for the detection of fish freshness (Ghosh *et al.* 1998). Nanodevices that consist of an array of nanosensors that are extremely sensitive to gases released by food have been included in food packaging; as the food spoils, the sensor strip changes color giving a clearly visible signal of whether the food is fresh or not. Arshak *et al.* (2007) reported the use of an array of conducting polymer composite nanosensors containing both carbon black and polyaniline to detect and identify the foodborne bacterial pathogens such as *Salmonella* spp., *B. cereus* and *Vibrio parahaemolyticus* through production of an individual response pattern for each bacterium. Their work demonstrates the potential application for the on-site identification of foodborne pathogens where these nanosensors could be interfaced with handheld devices to quantify emissions emanating from samples of contaminated foods.

17.3.2   Toxin sensors

As toxins are potent biomolecules causing pathogenesis in a wide range of populations, quick identification of these agents is critical. Frequent recalls of produce at a global level have occurred recently, resulting in severe economic losses and damaging international trade relations (Chaudhry and Castle, 2011).

Among the most prominent and affordable nanotechnology-based toxin detection venues are colorimetric assays that utilize nanoparticle probes. For instance, using thiolated lactose derivatives and gold nanoparticles, researchers were able to detect cholera toxin through molecular mimicry, as the nanoparticles' coating resembled the extracellular matrix terminal portion of GM1 ganglioside which is found in the apical membrane of intestinal epithelial cells (Schofield *et al.*, 2007). Detection and quantification were achieved visually and spectrophotometrically through color changes in the nanoparticle suspension (red to deep purple) and shifts in the nanoparticles' plasmonic band, as increases in the toxin concentration facilitated concomitant red shifts in the UV–visible absorption spectra (Schofield *et al.*, 2007).

Since portability is a key element for the detection of toxins, antibody-carrying gold nanoparticles have been immobilized to immuno-chromatography strips, allowing the visual detection of aflatoxins in grain samples (Shim *et al.*, 2007). Electrochemical detection is another popular method by which nanomaterial-based sensors with applications in the food industry function. Compared to optical (colorimetric or fluorimetric) methods, electrochemical approaches may be more useful for food matrices because the problem of light scattering and absorption from the various food components can be avoided. Many electrochemical sensors operate by binding selective antibodies to a conductive nanomaterial (e.g. carbon nanotube) and then monitoring changes to the material's conductivity when the target analyte binds to the antibodies.

17.3.3   Food traceability systems

Safety through traceability can be assured by the coupling of the existing technologies of (a) global positioning (GPS); (b) bar/chip coding; and (c) hazard analysis critical control point (HACCP) management, coupled to the on-going development of rapid (minutes and seconds) nanotechnology-based marker assays (Lachance, 2004). The coupling of these technologies provides scientific underpinning as well as global sourcing management, assuring standardization data for quality assurance of safety and efficacy, enhancing manufacturer accountability, and enabling highly rapid and effective recalls. Safety is the goal of commercial viability and marketability (Das *et al.* 2011).

Barcode technology provides accountability as well as data. Sophisticated barcode readers with chip technology are commercially available. With nanotechnology, the incorporation of chip capability within the consumer barcode is possible. The barcode should include reference to the Tier One technology files as well as the HACCP files that constitute Tier Three technology, yielding intelligent product delivery systems as described by Lachance (2004).

Barcoding has become a universal method for identifying products and their manufacturer, and barcoding is recognized by all consumers at the checkout counter. These barcodes can be used to identify the product source, various key dates, HACCP data, and related files. Nanotechnology can help food industries in proving authentication, and track and trace features of a food product to avoid counterfeiting and prevent adulteration and diversion of products destined for a specific market or contamination after processing. To help with tracking and tracing, nanotechnology provides complex invisible nanobarcodes with batch information which can be encrypted directly onto the food products and packaging. This nanobarcode technology offers food safety by allowing the brand owners to monitor their supply chains without having to share company information to distributors and wholesalers (Neethirajan and Jayas, 2011).

NanoInk, Skokie, US have developed a patterning technique called Dip Pen Nanolithography to encrypt information directly onto food products or pharmaceutical pills and on packaging (Zhang *et al.* 2009). The technique involves using a scanning probe molecule-coated tip to deposit a chemically engineered ink material to create a nanolithographic pattern on the food surface. Authentix, Addison, US have developed and are marketing nanoscale markers that can be incorporated into product packaging.

Nam *et al.* (2003) have made nanodisks of gold and nickel to encrypt information to be used as biological labels in applications such as DNA detection and as tags for tracking food products. The nanodisks were functionalized with dye molecules called chromophores that emit a unique light spectrum when illuminated with a laser beam. Li *et al.* (2007) have created a nanobarcode detection system that fluoresces under ultraviolet light in a combination of color that can be read by a computer scanner. Food and biological samples containing various combinations of *E. coli*, anthrax, and tularemia bacteria and Ebola and SARS viruses have been tested using this system and several pathogens were simultaneously distinguished by different-colored codes.

17.4 Safety regulations regarding food-applied nanotechnology

The potential risks arising from nanoscience and nanotechnologies in food and feed are being observed by international regulatory agencies, who consider that the current risk assessment paradigm is appropriate for nanomaterials. However, there are limited data on oral exposure to nanomaterials and any consequent toxicity; there are limited methods to characterize, detect, and measure nanomaterials in food/feed. Toxicological and toxicokinetic profiles of nanomaterials cannot be determined by extrapolation from data on their equivalent non-nano forms. A case by case approach is needed.

One draft guidance document that addresses the use of nanotechnology by the food industry was issued by the US Food and Drug Administration (FDA, 2012). The food draft guidance describes the factors manufacturers should consider when determining whether changes in manufacturing processes, including those involving nanotechnology, create a significant change that may: affect the identity of the food substance; affect the safety of the use of the food substance; affect the regulatory status of the use of the food substance; or warrant a regulatory submission to FDA. To conduct safety assessments for cosmetic products containing nanomaterials, standard safety tests may need to be modified or new methods developed. The guidance encourages manufacturers to consult with the agency before taking their products to market. Such consultation can help FDA experts address questions related to the safety or other attributes of nanotechnology products, or answer questions about their regulatory status. Strong science is critical to FDA's ongoing review of the products it regulates. FDA is investing in an FDA-wide nanotechnology regulatory science program to further enhance the scientific capabilities of the FDA, including developing necessary data and tools to identify properties of nanomaterials and assess the impact they may have on products.

The UK Food Standards Agency (FSA) published a review that considered the regulatory implications and risk assessment in relation to applications of nanotechnologies in food (FSA, 2012). The assessment concluded that food/food-packaging applications of nanotechnology will be subject to some form of approval process before being granted permission for use. However, they have also highlighted the general lack of knowledge in relation to potential consumer health risk regulatory aspects relating to nanoscale food additives.

The use of food additives in the EU is controlled by European Parliament and Council legislation and is based on the principle that only additives that are explicitly authorized may be used in food. Legislation most relevant to

nanoscale food additives is provided under Framework Directive 89/107 and the subordinate legislation. Nanofood additives are assessed either as novel additives or, where a macro-equivalent is already approved, through potential amendments of the appropriate specifications, including purity criteria, under Directive 96/77/EC. It is clear from the assessment of relevant EU legislation that most applications of nanotechnology in food and food contact materials (FCMs) will be subject to some form of approval process before being granted permission for use. However, it is also clear that current legislation pertaining to food ingredients, food additives, and FCMs does not differentiate between substances produced routinely by 'standard' manufacturing methods and those developed by nanotechnology. For example, current legislation does not differentiate between conventional and nano forms of food additives already approved for use in food.

17.5   Conclusions and outlook

A major area of current research is directed towards developing new and improved food-safety-assuring materials. In this regard, polymer nanocomposite films incorporating nanoparticles, nanosensors, or antigen-detecting biosensors are being developed for use in this area. When available, the embedded sensors in a packaging film will be able to detect food-spoilage organisms and trigger an alert to the consumer that the shelf life is ending or its safety is compromised. Nanoscale-sensing devices are also under development that, when attached to food products and packaging, will enable the food or food ingredients to be traced back to the source of origin or detect contamination.

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18
Photonic Methods for Pathogen Inactivation

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Summary

Photonic methods are among the non-thermal methods for increasing food safety. They have the use of light to inactivate microorganisms in common, and also have applications for decreasing food allergenicity and decomposing mycotoxins. Continuous UV light, pulsed light and photosensitization are the main photonic methods. This chapter describes their principle microbial inactivation mechanism and their application to fruit and vegetables, meat and meat products, water and liquid foods and others.

18.1 Introduction

Photonic methods for foodborne pathogen inactivation refer to methods based on the inactivation of microorganisms by light. This chapter covers continuous-wave ultraviolet (CW UV) light, pulsed light (PL) and photosensitization. Photonic methods have been used to increase the safety of foods given their capacity to inactivate microorganisms. Nevertheless, recent advances have shown their utility to decrease the allergenicity of foods and

to decompose mycotoxins. The oldest photonic method for microbial inactivation is CW UV light. Its use in the disinfection of drinking water began in 1906 in Marseille, France (Masschelein and Rice, 2002) and dates back to 1916 in the United States (EPA, 1996).

Ultraviolet (UV) light is the part of the electromagnetic spectrum with wavelengths between 100 and 400 nm, which includes UV-C light ($\lambda = 200$–280 nm) (Figure 18.1). The UV-C part
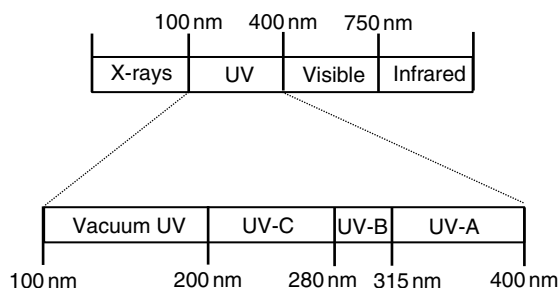
Figure 18.1 Electromagnetic spectrum with special emphasis on UV light.

of the spectrum is the most relevant for food safety due to its well-established antimicrobial capacity. The classical source of UV-C light is the low-pressure mercury lamp, which emits a quasi-monochromatic output at $\lambda = 253.7$ nm. More efficient medium-pressure lamps emitting polychromatic output began to be used later (Bolton and Linden, 2003).

UV-C light is non-ionizing. Since gamma-irradiated products have had marketability problems due to rejection by some consumers, the term 'illumination' (Lagunas-Solar and Gómez-López, 2006) will be used, and terms such as 'UV radiation' or 'UV irradiated' are evaded in order to avoid misconceptions.

Pulsed light (PL) is a non-thermal emerging technology to decontaminate surfaces and transparent liquids by inactivating microorganisms using pulses of an intense broad-spectrum (from infrared to UV) light where its UV-C part is the most lethal (Gómez-López *et al.*, 2007). It is characterized by the use of an extremely intense light that is delivered to the target in fractions of seconds several times per second, and is capable of inactivating microorganisms much faster than CW UV light and other thermal and non-thermal methods.

Photosensitization is a treatment involving a photoactive compound that accumulates in microorganisms and is followed by illumination with visible light (Lukšiene, 2005).

18.1.1 Dosimetry

The unit used by the International Union of Pure and Applied Chemistry to describe UV treatments is fluence ($J m^{-2}$) (Verhoeven, 1996), the light energy incident on food surfaces. For PL, the unit $J cm^{-2}$ is widespread. The US FDA has set $12 J cm^{-2}$ as the maximum allowed fluence for the treatment of foods with PL (FDA, 1996). Reporting treatment conditions in fluence or related terms allows comparisons between results from different sources and helps scaling up, but some authors tend to report treatment time or number of pulses. Good dosimetry requires special devices and knowledge of light properties; for a deeper insight on the subject Bolton and Linden (2003) is an excellent reference.

An important law governing photochemical processes is the Bunsen–Roscoe reciprocity law, even though some deviations to this rule have been identified. The principle of Bunsen–Roscoe affirms that, for the effectiveness of radiation, it is equivalent whether the energy required for the photochemical process is reached by using low fluence rate and long exposure time (as in CW UV light treatment) or high fluence rate and short exposure time (PL). The fluence of a treatment is obtained by multiplying fluence rate ($W cm^{-2}$) and exposure time (s).

18.2 Comparison of CW UV and PL treatment

18.2.1 Advantages and disadvantages of CW UV light

The use of CW UV light illumination for the treatment of foods is in general terms easy to apply and inexpensive. CW UV light has broad antimicrobial action, being capable of effectively inactivating viruses (Eischeid *et al.*, 2009) to parasites (Hijnen *et al.*, 2006). As microbicidal, it can substitute the application of chemical sanitizers which, together with the non-formation of byproducts, brings about benefits for the safety of the food and also of the environment. It is safe to use, although it requires certain precautions. Its use in the water industry was instigated by the discovery that it is effective for the inactivation of chlorine-recalcitrant parasites *Cryptosporidium parvum* and *Giardia* sp. (Hijnen *et al.*, 2006).

The Grotthuss–Draper law, also known as the first law of photochemistry, states that light must

be adsorbed by a compound in order for a photochemical reaction to take place. Since UV light is readily absorbed by the surface of foods, its action is limited to their outer layers or to transparent liquids, and its efficacy is severely impaired by shadow effects of other pieces of foods or even the same piece. Complexity and cost of the equipment can increase when special designs have to be used to avoid overlapping food pieces, to deliver light to the whole surface of big tridimensional food bodies such as oranges or when the treatment of non-transparent liquids requires thin layers and/or optimized hydraulics.

The application of UV light requires simple (but effective) safety precautions to avoid exposure of workers to light and to exhaust the ozone generated by the lowest UV wavelengths. Another disadvantage is that it is necessary to warm up lamps before use.

18.2.2   Advantages and disadvantages of PL compared to CW UV light

Pulsed light shares the advantages and disadvantages of the UV light itself with CW UV light. The advantage of PL over other microbial inactivation methods is its speed; depending on the characteristics of the PL device, it can achieve high inactivation levels in seconds. It can inactivate a wide range of microorganisms from bacteria to parasites and also viruses very quickly; a single pulse is able to cause more than 8 log reductions in less than a second to *in vitro* cultures.

PL is much faster that CW UV light (Gómez-López, 2012b). Comparisons of the efficacy of CW UV light and PL are made in experiments where fluence is kept constant, and have demonstrated the superiority of the latter in terms of inactivation rate and fluence microbicidal efficacy. Levy *et al.* (2012) reported that the inactivation of spores of *Bacillus subtilis* and other *Bacillus* spores at the same fluence was equivalent for both methods but 200,000 times faster with PL. Violations of the Bunsen–Roscoe principle were detected because spores of *Geobacillus stearothermophilus* and *Aspergillus niger* were more efficiently inactivated by PL; in fact, almost no inactivation was registered for *A. niger* spores subjected to CW UV light while a 5-log reduction was registered with PL (Figure 18.2).

Bohrerova *et al.* (2008) compared the disinfection efficiency of PL and CW UV light over *E. coli* cells and phages T3 and T4, which were all inactivated more efficiently by PL at equivalent fluence levels. For example, in order to achieve 3-log reduction of *E. coli*, a fluence of $5.1\,mJ\,cm^{-2}$ was required from CW UV light versus $2.2\,mJ\,cm^{-2}$ from PL.
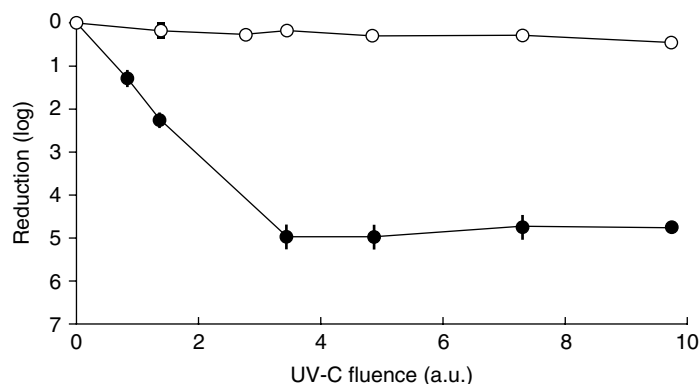
Figure 18.2   Inactivation of **Bacillus subtilis** spores on polystyrene as function of the UV-C fluence of CW UV light (o) and PL (•). a.u.: arbitrary units. Reprinted from International Journal of Food Microbiology, 152, Levy, C., Aubert, X., Lacour, B. and Carlin, F. Relevant factors affecting microbial surface decontamination by pulsed light, 168–174, Source: Levy *et al.*, 2012. Reproduced with permission of Elsevier.

PL penetrates deeper than CW UV light. However, this fact must not be overestimated. The fluence decreased by 60% at a depth of 10 mm in food model systems (agar and whey protein) (Bialka *et al.* 2008b). It can penetrate sausages, but 90% of the fluence is lost at a depth of 2.5 mm (Uesugi and Moraru, 2009).

Other advantages of PL over CW UV light is that PL (xenon) lamps do not require warming up and its modules are usually easily installable in pre-existing processing lines (Gómez-López, 2012a).

Among the disadvantages, food heating is an unwanted side effect of PL that must be assessed. Monitoring temperature during experiments with PL allows simple thermal effects on the observed inactivation to be ruled out, although most of the scientific reports show that food overheating is rare. Another disadvantage is the high cost of PL devices, although the fact that some industries have already implemented this technology demonstrates its economic feasibility for certain applications.

18.2.3   Inactivation of microorganisms and viruses *in vitro*

The inactivation of microorganisms by CW UV light is an accepted and useful application. For example, viruses are very susceptible to UV light. Illumination of less than $0.1 \, \mathrm{J \, cm^{-2}}$ is needed to achieve 4 log inactivation of cell-free feline calicivirus, murine norovirus and echovirus 12 (tested as surrogates of human norovirus) and even intracellular echovirus 12. In particular, cell-associated intracellular echovirus 12 was approximately three times more resistant to UV light than cell-free viruses (Park *et al.*, 2011).

As for PL, the first experiments on microbial inactivation were performed *in vitro*. The pioneer work by Bank *et al.* (1990), complemented by Bank (1992), demonstrated the efficacy of this technology for bacterial inactivation. The susceptibility of foodborne pathogens was later tested by MacGregor *et al.* (1998) showing reductions higher than 7 log CFU/plate for *E. coli* O157:H7 and *Listeria monocytogenes*. This technique was later shown to inactivate other foodborne pathogens such as *Salmonella* Typhimurium, *Shigella*, *Yersinia enterocolitica*, *Bacillus cereus* and *Clostridium perfringes* (Gómez-López *et al.*, 2005a).

PL is also very effective to inactivate viruses as demonstrated by an inactivation > 4 for polio and rotavirus in water (Huffman *et al.*, 2000). Roberts and Hope (2003) reported that a single pulse of $1.0 \, \mathrm{J \, cm^{-2}}$ decreased levels of 6 out of 9 viruses suspended in a buffer below the detection limit, with reductions up to 7.2 log. Less than $1 \, \mathrm{J \, cm^{-2}}$ was needed by Lamont *et al.* (2007) to inactivate poliovirus and adenovirus in suspension by approximately 4 log, and by Jean *et al.* (2011) to completely inactivate (5 log cycles) murine norovirus-1 (used as surrogate for human norovirus) and hepatitis A virus in suspension and on the food-contact surface materials (stainless steel and polyvinyl chloride).

PL can also inactivate yeasts and conidia (Gómez-López *et al.* 2005a) and mould spores; *Aspergillus niger* spores inoculated in corn meal have been reduced by 4.95 log CFU $\mathrm{g^{-1}}$ (Jun *et al.*, 2003). It has attracted the attention of water treatment researchers because it can inactivate *Cryptosporidium parvum*, a parasite of health concern which is very resistant to chlorine. At a fluence of $0.5 \, \mathrm{J \, cm^{-2}}$, PL can reduce *C. parvum* population in water >4 log (Huffman *et al.*, 2000). PL has also been capable of reducing *C. parvum* oocyst viability by 4.9 log and infectivity by 6.0 log when a fluence of $0.28 \, \mathrm{J \, cm^{-2}}$ is used (Lee *et al.*, 2008). Furthermore, Garvey *et al.* (2010) reported ≥4 log reduction in the infectivity degree of *C. parvum*.

18.3   Microbial inactivation mechanism

18.3.1   Continuous UV light

The formation of cyclobutane thymine dimmers in bacterial DNA is the main lethal event caused by UV-C light. The formation of covalent links between adjacent bases due to
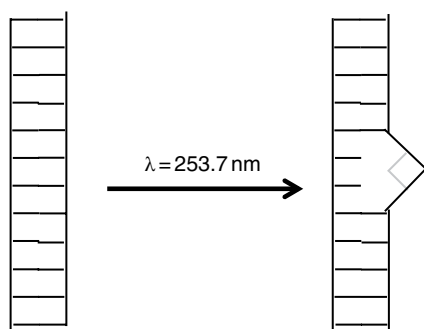
Figure 18.3 DNA molecule without (left) and with (right) a cyclobutane thymine dimmer.

exposure of DNA to UV light (Figure 18.3) inhibits the generation of new DNA, resulting in the inability to replicate the affected microorganism (Harm, 1980). UV-C treatment results mainly in formation of the 'spore photoproduct' 5-thyminyl-5,6-dihydrothymine on bacterial spores (Varghese, 1970). CW UV light is effective at inactivating microorganisms because low-pressure mercury lamps, the classical UV light source, emit a quasi-monochromatic output at the wavelength of 253.7 nm; this is close to 260 nm, the peak absorbance of DNA. Other light sources such as medium-pressure mercury lamps or xenon lamps emit a wide spectrum, which includes 260 nm.

The damage inflicted by UV-C light to cells can be repaired by microorganisms in different ways. Photoreactivation is a faster and more important mechanism that consists of the cleavage of the pyrimidine dimmer restoring the individual components by the enzyme photolyase, which harnesses blue or near-UV light energy to catalyse the reaction (Cleaver, 2003; Kao *et al.*, 2005). The dark repair mechanism or nucleotide excision repair is a slow enzymatic mechanism independent of light (Zimmer *et al.*, 2003). Photoreactivation has food safety implications because inactivated microorganisms can recover if exposed later to visible light. Spores can repair its DNA damaged by CW UV from spore photoproduct by common excision repair, or by the spore photoproduct specific repair system (Setlow, 1992).

18.3.2 Pulsed light

The microbial inactivation by PL is explained based on three effects: the photochemical, the photophysical and the phothermal effects. These effects are in line with the view stated by Wuytack *et al.* (2003) that PL microbial inactivation is a multitarget process.

18.3.2.1 Photochemical effect

PL has the same effect on DNA as CW UV light. The formation of cyclobutane thymine dimmers caused specifically by PL was demonstrated by Takeshita *et al.* (2003) in *Saccharomyces cerevisiae* and Bohrerova *et al.* (2008) in *E. coli*. The determination of the number of thymine dimers in the *E. coli* DNA showed a higher number after PL than after CW UV light treatment (Bohrerova *et al.*, 2008). The main effect of PL is mainly, but not exclusively, the UV-C light. For *E. coli*, the higher number of dimmers was due to wavelengths >295 nm; for viruses, the high intensity of wavelengths >400 nm might be responsible for some additional physical damage such as phage capsid rupture (Bohrerova *et al.*, 2008).

As in CW UV light, microbial DNA can undergo photoreactivation, as demonstrated by Otaki *et al.* (2003) for *E. coli*, Gómez-López *et al.* (2005a) for *L. monocytogenes* on agar plates and for *Salmonella* Enteritidis on shell eggs (Hierro *et al.*, 2009), although photoreactivation rates were lower in flashed cells (Otaki *et al.*, 2003). This finding might be related to damage of the photoreactivation enzymes. On the other hand, no photoreactivation was observed by Paskeviciute *et al.* (2011) for *Salmonella* Typhimurium and *L. monocytogenes* on agar plates and by Hierro *et al.* (2011) for *L. monocytogenes* inoculated on vacuum-packaged cooked ham and bologna slices.

18.3.2.2 Photophysical effect

The photophysical effect refers to the structural damage in microbial cells attributed to the constant disturbance caused by the high-energy pulses. This kind of damage in *Saccharomyces cerevisiae* cells consists of a loss of cell membrane permeability, detected by a higher concentration

of eluted protein and microscopic observation of raised and expanded vacuoles, cell membrane distortion and modification of cell shape; these changes are not observed in cells illuminated by CW UV light (Takeshita *et al.*, 2003). In *Staphylococcus aureus* cells leakage, lack of cell wall, cytoplasmic membrane shrinkage and collapse of internal structures occur (Krishnamurthy *et al.* 2010).

18.3.2.3   Photothermal effect

The photothermal effect consists of the inactivation of microbial cells by an elevation of the temperature due to absorption of PL rays directly or by conduction from their surroundings. Despite the increase in temperature, PL should still remain a non-thermal method since any heating of the food is restricted to its most superficial layer and is quickly dissipated, causing only a very limited overall increase of food temperature. The first mechanism was used by Hiramoto (1984) to explain the inactivation of *Aspergillus niger* by PL, while the second was used by Dunn *et al.* (1989).

Micrographies of spores of *A. niger* presenting severe deformation and rupture have been presented as evidence of cell inactivation attributed to momentous overheating caused by PL (Wekhof *et al.* 2001). Similar results were reported by Nicorescu *et al.* (2013) in *Bacillus subtilis* vegetative cells inoculated on species and treated at $6\,J\,cm^{-2}$ (Figure 18.4). Other authors have not observed such effects, however. Levy *et al.* (2012) treated *Bacillus subtilis* and *A. niger* spores with $3.6\,J\,cm^{-2}$, observing no disruption in spore structure. In virus, Bohrerova *et al.* (2008) suggested that besides DNA damage, phage capside might be ruptured by the visible portion of PL.

18.4   Sublethal injury, acquired resistance and sensitization

A microbial cell subjected to inactivation treatments can survive either uninjured or injured. When grown in selective media, this mixture of cells can lead to an underestimation of the number of survivors and consequently an overestimation of the safety of the food. The existence of sublethal injury after PL treatment was reported by Wuytack *et al.* (2003). Sublethal injury of PL-treated *Listeria monocytogenes* cells

(a)

(b)
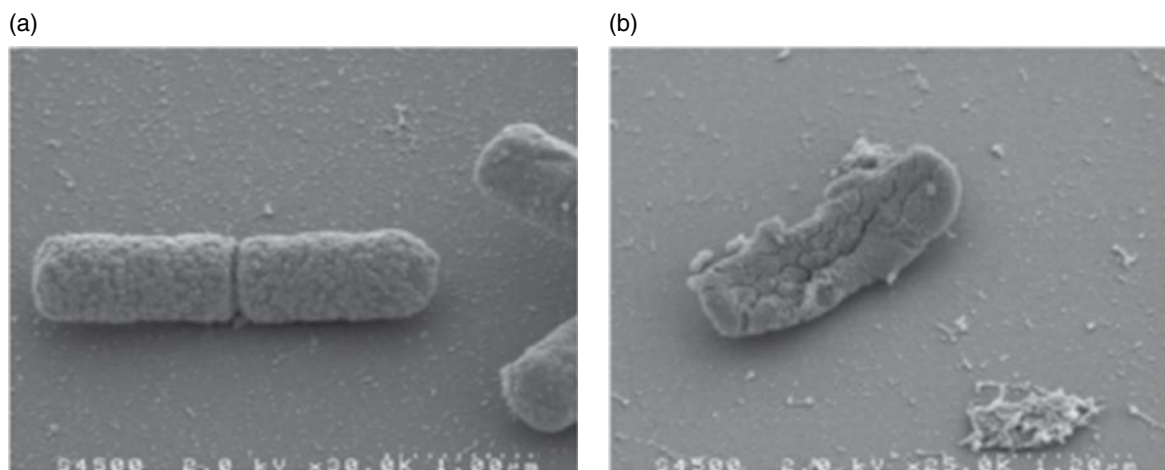
Figure 18.4   SEM of ***Bacillus subtilis*** vegetative cells: (a) untreated samples and (b) treated by $10\,J\,cm^{-2}$. Reprinted from Food Control, 31, Nicorescu, I., Nguyen, B., Moreau-Ferret, M., Agoulon, A., Chevalier, S. and Orange, N. Pulsed light inactivation of ***Bacillus subtilis*** vegetative cells in suspensions and spices, 151–157. Source: Nicorescu *et al.*, 2013. Reproduced with permission of Elsevier.

is expressed by a longer lag-phase with no effect on the maximum growth rate, which decreases with increasing levels of carbon dioxide (Van Houteghem *et al.*, 2008). This bacteria exhibits 40–100% of sublethally injured cells depending on the strain and with high variability among strains (Rajkovic *et al.*, 2009a). The proportion of injured cells can be about 90%, as reported for *Escherichia coli* and *Listeria innocua* suspended in apple juice and treated at $4\,J\,cm^{-2}$ (Pataro *et al.*, 2011).

Injured cells can develop resistance. The development of resistant pathogenic bacteria is a concern for the food industry, since a recalcitrant microflora can develop in their installations. Development of resistance is observed at the laboratory scale by a reduction of inactivation when microorganisms are treated with sublethal doses of the lethal agent and survivors recovered, cultured and treated again for several cycles. A higher number of survivors with increasing cycles is taken as an increased resistance. Using this approach, Gómez-López *et al.* (2005a) found that after 13 cycles *L. monocytogenes* did not show decreased inactivation levels, as was also observed by Elmnasser *et al.* (2007) for *Pseudomonas fluorescens*, *Photobacterium phosphoreum* and *L. monocytogenes* after 3 cycles, using a single pulse of $1.5\,J\,cm^{-2}$. On the other hand, increased resistance of 1 and 2 log CFU mL^{-1} were reported for *L. monocytogenes* and *Escherichia coli* O157:H7 respectively after 20 cycles (Rajkovic *et al.*, 2009b) with only one strain from the initial cocktail surviving that number of cycles, which showed a longer lag-phase. Using a different experimental approach, the development of resistance to PL by *Enterococcus faecalis* has been related to the increase of bacterial mutation frequency and affectation of the abundance of 19 proteins (Massier *et al.*, 2012). There is no evidence of cross-resistance of PL-resistant variants of *L. monocytogenes* and *Escherichia coli* O157:H7 to low pH and heat (Rajkovic *et al.*, 2011).

On the other hand, previous treatments can increase the sensitivity of cells to PL. The influence of mild conventional food processing stress treatments (use of salt 7.5% for 1h, acid pH 5.5 for 1h) on survival and virulence-associated characteristics of *L. monocytogenes* has been studied by Bradley *et al.* (2012). Their results showed that prior exposure to some food processing stress conditions increases sensitivity of *L. monocytogenes* to PL.

18.5 Kinetics of microbial inactivation

The use of non-log-linear models to describe the kinetics of microbial inactivation has increased in the last years; this is a closer approximation to reality instead of trying to fit results to Chick's law curves that are clearly non-log-linear. The availability of software and add-in tools such as GInaFiT (Geeraerd *et al.*, 2005, 2006) have allowed non-kinetic experts to test the adjustment of their data to a wide variety of kinetic models.

The basic non-log-linear inactivation curve typical of CW UV light inactivation has a sigmoidal shape where a shoulder, a log-linear phase and a tail can be distinguished (Sastry *et al.*, 2000). The Weibull model is however the most commonly reported for PL inactivation; showing an upward concavity, it has only a log-linear phase and a tail. It has been reported for *Listeria innocua* on inert surfaces (Uesugi *et al.*, 2007); *Salmonella enterica* and also *Escherichia coli* O157:H7 (Bialka *et al.*, 2008a) on raspberries and strawberries; *E. coli* O157:H7 and a non-pathogenic *E. coli* strain inoculated in apple juice and apple cider (Sauer and Moraru, 2009); natural microflora of fresh-cut iceberg lettuce, white cabbage and Julienne-style carrots (Izquier and Gómez-López, 2011); and *S*. Typhimurium on chicken breast and *L. monocytogenes* on chicken frankfurters (Keklik *et al.*, 2012).

The Weibull model takes the following form when adapted as a function of fluence:

$$\log\left(\frac{N}{N_0}\right) = -\alpha F^{\beta}$$

where N is the number of survivors after applying a fluence F; N_0 is the initial population; α is the scale parameter; and β is the shape factor (Sauer and Moraru, 2009). Tailed curves have $\beta < 1$, which means an upward concavity.

Nevertheless, a complete sigmoidal curve has been reported for the PL inactivation of *S. Enteritidis* on shell eggs (Keklik *et al.*, 2012), by Marquenie *et al.* (2003) for *Botrytis cinerea* and *Monilia fructigena* in buffer and by Levy *et al.* (2012) to describe the inactivation of cells and spores of different *Bacillus* species and other microorganisms on inert substrates. It is not clear why the shoulder appears in some microorganism-matrix pairs and not in others.

Tails might occur in bacteria present on food surfaces due to the shielding provided by surface irregularities (Bialka *et al.*, 2008a). As for liquid substrates, suspended particles and cell aggregates can also shield microorganisms as also occurs in CW UV treatments (Unluturk *et al.*, 2008); furthermore, the probability of different targets to be reached by photons is reduced with decreasing population density (McDonald *et al.*, 2000). No tail but complete inactivation was reported for the inactivation of *Staphylococcus aureus* inoculated in milk and treated with PL in a flow-through reactor (Krishnamurthy *et al.*, 2007), but this was an exception. Other inactivation curves reported for PL show tailing, which has important implications for food safety as this implies that a residual population will always remain in the food.

18.6   Application of photonic methods

18.6.1   Application to foods of vegetable origin

18.6.1.1   Continuous UV light
Viruses inoculated on produce surfaces can be inactivated by CW UV light. The inactivation of feline calicivirus (a surrogate for norovirus), hepatitis A virus and Aichi virus was within the range 4.5–4.6, 2.5–5.6 and 1.9–2.6 log $TCID_{50}$ mL^{-1} for lettuce, green onions and strawberries, respectively, when a low-pressure UV lamp was used to supply $0.24\,J\,cm^{-2}$. Virus inactivation was reported as 50% tissue culture infective doses ($TCID_{50}$) (Fino and Kniel, 2008).

Foodborne pathogens inoculated onto vegetables are also susceptible to CW UV light. This method has reduced *Salmonella* spp. populations by 2.19 and 2.65 logs in tomato and green leaf lettuce, respectively, and *Escherichia coli* O157:H7 populations by 2.79 and 3.3 logs on green leaf lettuce and apple surface (Yaun *et al.*, 2004). In minimally processed pears, the application of $87\,J\,cm^{-2}$ has caused the inactivation of *Listeria monocytogenes* by >3 logs (Schenk *et al.*, 2008). In baby spinach, the application of $2.4–24\,J\,cm^{-2}$ caused the inactivation of *Listeria monocytogenes* and *Salmonella* spp. by 1.5–2.2 and 0.7–1.6 log CFU g^{-1} (Escalona *et al.*, 2010). In Roma tomatoes and jalapeno pepper, $4\,J\,cm^{-2}$ inactivated *Salmonella* spp., *Staphylococcus aureus* and *Listeria monocytogenes* by >4 log CFU g^{-1} (Sommers *et al.*, 2010). CW UV light was found superior to treatments such as electrostatic sprays of electrolysed oxidizing water and ozone to decrease *Escherichia coli* O157:H7 populations on the calyx and skin of blueberries by 2.14 and 4.05 logs (Kim and Hung, 2012).

18.6.1.2   Pulsed light
The decontamination of alfalfa seeds with PL from *E. coli* O157:H7 has been tested together with its effect on seed viability; in an inactivation of >4 log CFU g^{-1} with 74% of seed viability was observed (Sharma and Demirci, 2003). The inactivation of mesophilic microorganisms of different minimally processed vegetables, namely spinach, green bell pepper, radicchio, grated carrot, iceberg lettuce and white cabbage, was within the range 0.56–2.04 log (Gómez-López *et al.*, 2005b).

The application of PL to small fruits has also been studied. In raspberries, the maximum inactivation was 3.9 log CFU g^{-1} for *E. coli* O157:H7 at $72.0\,J\,cm^{-2}$, with no observable damage to the fruit. In strawberries, the results reported are 2.1 log CFU g^{-1} for *E. coli* O157:H7 and 2.8 log CFU g^{-1} for *Salmonella* at 25.7 and $34.2\,J\,cm^{-2}$ respectively,

treatments that do not cause damage of the calyx of the fruit. Higher inactivation levels were observed at upper fluences but with significant impairment of fruit quality (Bialka and Demirci, 2008). The same kind of result was reported by Bialka and Demirci (2007) for blueberry, log reductions for *E. coli* O157:H7 and *Salmonella* of 4.3 and 2.9 log CFU g^{-1} without observable fruit damage; higher inactivation at higher fluences damaged the fruit.

PL can also be used to increase the microbial safety of fresh-cut avocados; it can reduce the populations of *Listeria innocua* (surrogate of *L. monocytogenes*) by 3 log after a 12 J cm^{-2} treatment, although it induced fruit browning which could have been prevented by other means (Ramos-Villarroel *et al.*, 2011). The inactivation of bacteria in spices seems to be more limited, with no more than 1 log reduction of *Bacillus subtilis* vegetative cells inoculated on ground caraway, black and red pepper (Nicorescu *et al.*, 2013).

The data presented in this compilation must take into account the recent findings that bacteria can be internalized by produce in the field (Erickson, 2012). Once below the surface, the capacity of UV light (and many other decontamination treatments) to inactivate them is seriously limited.

18.6.2   Application to meat products

18.6.2.1   CW UV light
The efficacy of CW UV light to increase the safety of pork, chicken and sausages has been tested by different authors. The application of a treatment of 1.92 J cm^{-2} UV-C decreased *Escherichia coli* populations by 1.9 and 3.3 log CFU cm^{-2} and *Salmonella* Senftenberg by 2.0 and 4.6 log CFU cm^{-2} in pork muscle and skin surfaces, respectively (Wong *et al.*, 1998). A fluence of 4 J cm^{-2} inactivates *Salmonella* by 2.19, 1.51, 0.45, 0.32 and 0.53 log CFU g^{-1} on fat-free frankfurters, pre-cooked bratwurst, drumsticks, chicken breast and pork chop, respectively. It also inactivated *Listeria monocytogenes* by 2.14, 1.78, 0.63, 0.37 and 0.65 logs on those respective meat items, and *Staphylococcus aureus* by 1.97, 1.38, 0.42, 0.44 and 0.49 logs, respectively (Sommers *et al.*, 2010). A higher decontamination of chicken breast by UV-C treatment up to 0.5 J cm^{-2} has been found, reducing populations of *Campylobacter jejuni*, *Listeria monocytogenes* and *Salmonella* Typhimurium by 1.26, 1.29, 1.19 log CFU g^{-1} respectively (Chun *et al.*, 2010). Haughton *et al.* (2011) also reported the inactivation of *Salmonella* and *Campylobacter* in chicken fillet; a depiction of the UV treatment unit is provided in Figure 18.5. More interestingly, these authors observed variations up to 4 log CFU mL^{-1} in the inactivation of different *Campylobacter* isolates in preliminary tests in liquid media.

18.6.2.2   Pulsed light
The application of PL to meat products has been proved to have relatively low efficacy for surface decontamination. The reduction of *Escherichia coli* O157:H7 on salmon fillets has been reported to be 1.09 and 0.86 log CFU g^{-1} at muscle and skin sides, respectively, and 1.02 and 0.74 log CFU g^{-1} respectively for *Listeria monocytogenes*. However, surface
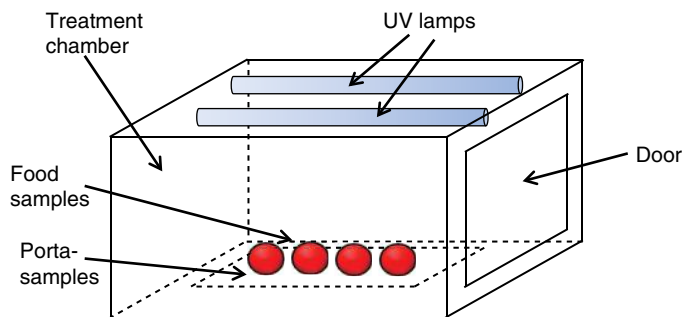
Figure 18.5   Schematic representation of a bench-top UV treatment unit.

temperature increased up to 100 °C, resulting in product quality impairment (Ozer and Demirci, 2006). Reductions in *S.* Typhimurium and *Listeria monocytogenes* populations on skinless chicken breast surface reached 2 log CFU after a 5.4 J cm^{-2} treatment (Paskeviciute *et al.*, 2011). In beef carpaccio, Hierro *et al.* (2012) reported decontamination from *E. coli* O157:H7, *S.* Thyphimurium and *Listeria monocytogenes* up to 1 log CFU cm^{-2} with 4.2 J cm^{-2}. At the same fluence, a reduction of 0.5 log CFU cm^{-2} was observed for *Vibrio parahaemolyticus* and *Listeria monocytogenes* in tuna carpaccio. Higher fluences produced greater inactivation, a maximum of 1.2 log CFU cm^{-2}, but affected colour and sensory quality negatively.

A reduction in *Listeria monocytogenes* populations on unpackaged and vacuum-packaged chicken frankfurters of 1.9 log CFU cm^{-2} (Keklik *et al.*, 2009a) and a reduction of *Listeria innocua* (a surrogate for *L. monocytogenes*) on ready-to-eat sausages of 1.37 log CFU per sausage after exposure to 9.4 J cm^{-2} was reported (Uesugi and Moraru, 2009). The inactivation of *L. monocytogenes* was 1.78 and 1.11 log CFU cm^{-2} on cooked ham and bologna slices, respectively, after 8.4 J cm^{-2}. This fluence tripled the shelf-life of vacuum-packaged cooked ham, but fluences above 2.1 J cm^{-2} modified the sensory quality of vacuum-packaged bologna slices (Hierro *et al.*, 2011).

It is possible that the limited decontamination observed in meat products might have been predicted from the conclusions of Gómez-López *et al.* (2005b), where the authors observed lower microbial inactivation levels at increasing concentrations of proteins and oil but no effect of water and carbohydrates. It was concluded that this method was inappropriate for proteinaceous and fatty foods.

18.6.3   Application to liquids

18.6.3.1   Continuous UV light
There are different types of reactors commercially available or under development for the treatment of fluids with UV light; designs include annular, thin film, coiled tube devices, static and dynamic mixers (Gómez-López *et al.*,

2012) and centrifugal reactors. Many designs try to minimize the attenuation effect of the liquid food by promoting specific fluid dynamics that can maximize microbial exposure to light. Those reactors usually yield very high inactivation levels and demonstrate the suitability of UV light to increase the safety of liquid foods. There are already examples of industrial applications in use such as the treatment of cider with CW UV light in New York State, in a, FDA-approved process (New York Apple Association, 2013).

Using a thin-film UV disinfection unit, it has been possible to reduce acid-adapted *Escherichia coli* O157:H7 in apple cider by 5.4 log CFU mL^{-1} by applying 0.061 J cm^{-2}; however, in order to achieve that fluence the flow rate was too low for practical application (Wright *et al.*, 2000). It was later demonstrated that apple cultivar and bacteria strain influence the inactivation (Basaran *et al.*, 2004). The commercial UV device CiderSure™ used to treat apple cider needed 0.014 J cm^{-2} to reduce the viability of *Cryptosporidium parvum* oocysts by >4.6 log (Hanes *et al.*, 2002). In liquid egg white, the inactivation of *Escherichia coli* treated by a coiled tube reactor was 4.3 log for 44 J mL^{-1} treatment (Geveke, 2008). In opaque liquids, 1.8 J mL^{-1} was unable to decrease *Mycobacerium avium* in milk, more than 1 log (Donaghy *et al.*, 2009), although *Bacillus cereus* spores inoculated in raw soymilk and subjected to 0.012 J cm^{-2} in a coiled tube UV reactor was inactivated by 3.3 log CFU mL^{-1} (Bandla *et al.*, 2012).

A centrifugal UV reactor consists of a core of UV lamp enclosed in a hollow stainless steel that rotates generating a thin film. Rotation speed and inclination of the reactor can be adjusted. This device was evaluated inoculating non-pathogenic microorganisms in grapefruit juice. A reduction of 5.1 log for *Escherichia coli* was reported after processing at 0.019 J cm^{-2}, 450 rpm and 15°, and a fluence of 0.014 J cm^{-2} reduced *Saccharomyces cerevisiae* populations by 6.0 log (Geveke and Torres, 2012).

18.6.3.2   Pulsed light
The application of PL to liquid foods is much newer than that of CW UV light; the sparse literature

therefore only describes experiments using very simple reactor configurations, yet with very relevant results. There are two kinds of experimental set-ups. One involves the use of Petri dishes as containers of inoculated liquids and bench-top systems to deliver the light, and the other is flow through reactors, closer to the way that UV technology can be applied in reality where fluid dynamics plays an important role.

Even although honey has a very low water content, it has been implicated in cases of botulism. *Clostridium sporogenes* is sometimes used as a surrogate of *Clostridium botulinum*. The use of PL to inactivate *C. sporogenes* in clover honey using a bench-top system has been studied. The rate of inactivation increased for longer treatment times, shorter lamp–sample distances and lower sample thicknesses, but did not reach >1 log CFU mL^{-1} reduction for any conditions. Some parameter combinations increased sample temperature up to 100 °C (Hillegas and Demirci, 2003).

The incorporation of shaking in experiments with bench-top devices is a step closer to real life. The application of PL to apple juice and cider to inactivate a non-pathogenic *E. coli* and the effect of turbulence were studied by Sauer and Moraru (2009). Liquid foods were placed in Petri dishes and turbulence promoted by orbital shaking. Inactivation levels under shaking were 5.49 and 7.29 log CFU mL^{-1} at 8.8 J cm^{-2} for cider and apple juice, respectively; under static conditions, inactivation levels of 3.2 and 2.7 log CFU mL^{-1} at 12.6 J cm^{-2} were observed. The results clearly show the positive effect of turbulence on the PL microbial inactivation. A higher inactivation (7.26 log CFU mL^{-1}), even reaching complete inactivation demonstrated by enrichment, was reported by Krishnamurthy *et al.* (2007) for *Staphylococcus aureus* inoculated in milk using a flow-through system of quartz tubes adapted to a conventional bench-top system. However, milk temperature rose as a consequence of the treatment.

A pilot-plant system was used by Chain *et al.* (2012) to test the inactivation of *Alicyclobacillus acidoterrestris* in 65° Brix sugar syrup. Even although this bacterium is not of public health concern, the results show the speed of the inactivation

by PL. Three-log reduction was achieved after 7 s of treatment (3 pulses with a pulse repetition rate of 0.4 s), which is shorter than the 20 min necessary to reach the same level of decontamination by heating at 95 °C. A pilot-plant unit for fluid treatment is shown in Figure 18.6.

A special method of PL treatment is the use of pulsed UV light excimer laser (248 nm). This technique was tested for the inactivation of *Escherichia coli* O157:H7, *L. monocytogenes*, *Salmonella choleraesuis*, *Yersinia enterocolitica*, *S. aureus*, *Aeromonas hydrophila* and *Serratia marcescens* inoculated in milk (Smith *et al.*, 2002). The system exposed milk samples placed in quartz cuvettes to energy of 25 J cm^{-2}, and no survivors were detected.

Koutchma (2009) has provided an excellent review on various aspects covering applications of UV in liquid food processing, critical product and process factors which affect UV inactivation including modelling of UV inactivation, effects of UV on nutritional and overall qualities of liquid foods and on the UV reactor designs that have been used for liquid foods treatment.

18.6.4   Application to other foods

CW UV light has been tested for decontamination of mushrooms inoculated with *Escherichia coli* O157:H7, reducing its population by 1.13 log CFU g^{-1} at 0.1 J cm^{-2} (Guan *et al.*, 2012). A small degree of inactivation was also recorded after a treatment of 4 J cm^{-2} of shell eggs, with logarithmic reductions of 0.98, 0.81 and 1.16 for *Salmonella* spp., *Staphylococcus aureus* and *Listeria monocytogenes* respectively (Sommers *et al.*, 2010).

PL is a highly efficient method for inactivation of *Salmonella* Enteritidis on the surface of shell eggs. A reduction of >5.3 log CFU cm^{-2} in *Salmonella* population was reported by Keklik *et al.* (2009b) with no growth after enrichment when 23.6 J cm^{-2} was applied. In the same line, Hierro *et al.* (2009) found that 80% of the eggs showed the maximum decontamination achievable in their experiments (3.6 log CFU/egg) after applying 12 J cm^{-2}.
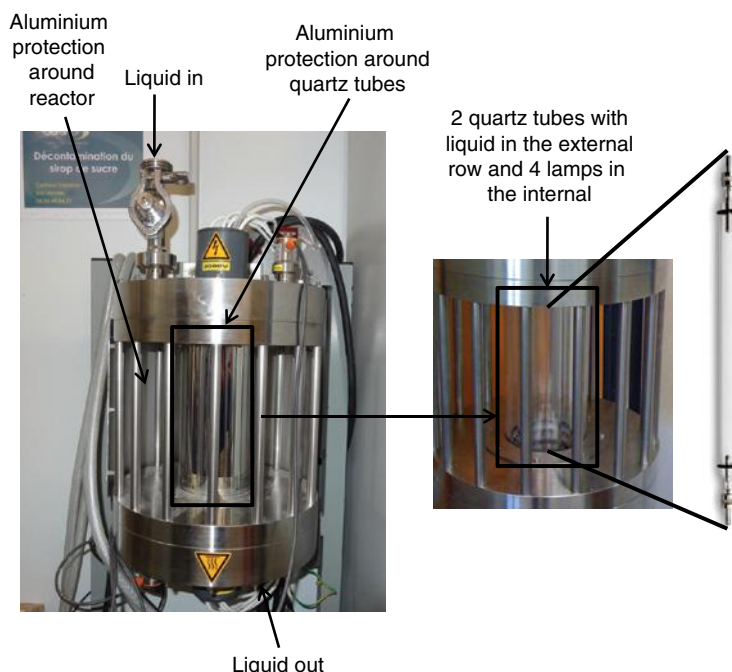
Figure 18.6   A pulsed right reactor for liquid treatment. Source: Claranor. Reproduced with permission of Claranor.

The inactivation of *L. monocytogenes* inoculated in two commercial infant foods spread as a 2 mm thick layer on Petri dishes has been reported by Choi *et al.* (2010). Inactivation levels were 5 log CFU g^{-1} in light-coloured thin beverage and 3 log CFU g^{-1} in dark-coloured viscous meal. A commercial PL fluidized bed system was used by Fine and Gervais (2004) for decontamination of powders using *Saccharomyces cerevisiae* as indicator microorganism, with >1 log reduction when the yeast was treated on black pepper at 16 J cm^{-2}. Other examples of the application of PL to foods are in curds of a dry (non-creamed) cottage cheese, hard-crusted white bread rolls, cake and raw shrimp (Dunn *et al.*, 1989).

Edible films can be used as carries of antimicrobials to enhance food safety. UV light has also been used in combination treatments with sugars (ribose and lactose as cross-linking agents) to improve the mechanical properties of fish gelatine films to be used either as edible films or for food packaging purposes (Bhat and Karim, 2012).

18.6.5   Decomposition of allergens by pulsed light

The use of PL in foods has been focused on microbial inactivation. However, recent studies have opened their application fields for food safety to new scenarios, such as reducing the allergenicity of some foods.

Peanut allergy is one of the most common food allergies. Eight major allergens have been identified in peanuts, among which Ara h 1, Ara h 2 and Ara h 3 are predominant (Yang *et al.*, 2012a). Based on the hypothesis that PL may cause protein insolubility like other high-peak-power technologies, which may lead to a reduction of peanut allergens, Chung *et al.* (2008) illuminated peanut extracts and liquid peanut butter at fluences of 220.8 and 165.6 J cm^{-2}, respectively. Their results showed that in fact PL insolubilizes peanut allergens due to aggregate formation, which opens the possibility for development of a hypoallergenic peanut product upon confirmation by clinical

studies of actual allergenicity reduction. However, the products lost 40% of their volume due to water evaporation. The reduction in allergenicity reported by Chung *et al.* (2008) was due to the effect of PL on Ara h 1 and Ara h 3. Later, Yang *et al.* (2012a) were able to inactivate Ara h 2, the most potent peanut allergen, after treating raw peanut extracts with 223.2 J cm^{-2}.

PL has also been shown to reduce the allergenic potency of shrimps. The most important heat-stable allergen in shrimps is tropomyosin. PL reduces the levels of tropomyosin and IgE binding against shrimp allergens after treating a shrimp extract for 4 min, which seems to be achieved at 194 J cm^{-2} according to the reported treatment conditions. Authors stated that this effect is likely due to cross-linking of tropomyosin (Shriver *et al.*, 2011). The possibility that the reduction in allergenicity caused by PL be restored during the transit of the allergen through the digestive system has been also tested. Yang *et al.* (2012b) performed *in vitro* gastric and intestinal digestions of PL-treated shrimps extracts, and found that the decrease in allergenicity caused by PL was maintained under human and digestive conditions; however, they state that their postulation must be verified with *in vivo* trials. PL has also shown to decrease the allergen potency of soybean extract, egg extract, milk and wheat extract according to Shriver and Yang (2011).

The success of the practical implementation of PL for allergen inactivation will depend on: its efficacy in actual foods which is limited by opacity; the harmonization between such high fluences and the limit set by the FDA; and on the effect on quality of the products, which could be impaired by water evaporation.

18.6.6 Decomposition of mycotoxins by pulsed light

One of the new applications of PL to reduce food safety risks is the degradation of mycotoxins. Moreau *et al.* (2011) destroyed 98, 93, 85 and 73% of ochratoxin, aflatoxin B1, zearalenone and deoxynivalenol by applying 8 flashes. The authors

demonstrated the fragmentation of ochratoxin and aflatoxin B1 by using mass spectrometry, and the complete elimination of mutagenicity in aflatoxin B1. They propose the use of PL for prophylactic surface treatment of products susceptible to carrying mycotoxins.

Patulin is a toxic secondary metabolite produced by fungi *Penicillium expansum* and is the most common contaminant in apples. PL has been proven to reduce patulin levels in buffer and apple products. The quantities of patulin remaining in a McIlvaine buffer after 35.8 J cm^{-2} was about 5–15%, while in apple juice it was about 22%. Temperature increases during treatment were observed, but not at levels that could account for the observed inactivation which is likely due to photochemical reactions. The remaining patulin in apple puree naturally contaminated by this mycotoxin fell below the detection limit at fluences higher than 11.9 J cm^{-2} and in artificially contaminated puree at fluences higher than 23.8 J cm^{-2}. The obvious influence of the food matrix has been attributed to the effect of ascorbic acid present in apple products. Despite these positive results, the study of toxicity of degradation products is pending (Funes *et al.* 2013).

18.6.7 Photosensitization

Photosensitization is a treatment involving a photoactive compound that accumulates in microorganisms and is followed by illumination with visible light. The combination of photosensitizer and light in the presence of oxygen results in the destruction of microorganisms (Lukšiene, 2005). The main pre-requisite for microbial inactivation by photosensitization is the accumulation of photosensitizers (i.e. haematoporphyrin, 5-aminolevulinic acid or Na-chlorophyllin) in the bacteria, which can be experimentally performed by incubation of bacteria for 20–30 min in a photosensitizer solution. The microorganisms are then illuminated with visible light for about 20 min.

There are considerable differences in sensitivity to this method between Gram-positive and

Gram-negative bacteria, with the latter less sensitive. As explained by Lukšiene and Zukauskas (2009), the photosensitizer accumulates in the cell wall of Gram-positive bacteria and yeasts, which is broken by the reactive oxygen species formed upon illumination. On the other hand, the outer membrane of Gram-negative bacteria rejects photosensitizers. The inactivation of Gram-negative bacteria by this method is still possible by promoting the generation of photosensitizers within the bacterial cell by incubation with a precursor (Buchovec *et al.*, 2009).

The inactivation of microorganisms by photosensitization *in vitro* or on packaging surfaces has yielded positive results for *Staphylococcus aureus* (Kreitner *et al.*, 2001), fungi (Lukšiene *et al.*, 2005), *Salmonella enterica* (Buchovec *et al.*, 2009), vegetative cells and spores of *Bacillus cereus* (Lukšiene *et al.*, 2010a) and *Listeria monocytogenes* (Lukšiene *et al.*, 2010b). The decontamination of strawberries inoculated with *Listeria monocytogenes* by chlorophyllin-based photosensitization yielded <1 log reduction, however (Lukšiene and Paskeviciute, 2011). The results are discouraging, but not surprising since it is well known that the rough surface of strawberries provides many sites were bacteria can shield from light; perhaps other foods will demonstrate higher decontamination yields.

18.7   Concluding remarks and future work

The study of photonic methods to increase food safety has gained importance in the last years. There exist applications of CW UV light technology already in use, while the study of PL applications has evolved from bench-top tests to pilot-plant reactors, with installed industrial devices for food contact surfaces. Photosensitization has produced promising *in vitro* results and new tests will reveal its real potential for increasing food safety. PL technology may be adapted to increase the safety of fruit juices with faster results than CW UV light, as long as the treatment is cost-effective. The unique properties of PL to inactivate food allergens deserves further study to establish its efficacy in actual foods in terms of inactivation, effects on quality and legal limits.

Acknowledgement

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19
Intelligent Packaging and Food Safety

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Summary

Intelligent packaging covers a variety of applications, where some element of intelligence is added to food. In particular, such packaging is designed to monitor and communicate information about food quality and safety and to provide greater convenience, improved traceability as well as enhanced logistical handling. Examples include time-temperature indicators, radio-frequency identification, spoilage or freshness indicators, ripeness indicators and biosensors. This chapter provides a brief overview of the various principles behind currently available intelligent packaging, including some representative examples. It also discusses the most relevant aspects related to advances and challenges in the use of such packaging. Nanotechnology-based sensing approaches are also addressed in the context of their potential future development for intelligent packaging applications.

19.1   Introduction

Significant changes in consumer preferences, increased regulatory requirements, globalization of markets, centralized activities and emerging concerns regarding food safety – all characteristics of the past few decades – have contributed in no small way to the development of novel packaging concepts with extended functionality. In particular, the major function of packaging has shifted from being a passive barrier against the adverse effect of the environment towards an active interaction between the food or the headspace and the packaging. In principle, active packaging (AP) is designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food with the aim of extending the shelf-life or to maintain or improve the condition of the food (European Commission, 2009).

Another paradigm shift that has taken place in recent years has been the progression of food packages from mediocre communicators to somewhat intelligent communicators, in other words, the emergence of intelligent packaging. Intelligent packaging (IP) enables the monitoring of and communication about food quality and safety and provides an early warning to the consumer or any member of the supply chain (Yam, 2012). In fact, a variety of IPs are suitable for monitoring freshness, the presence of pathogens, package integrity, carbon dioxide and oxygen content, pH, storage time and/or temperature. This technology also helps to trace a product's history through the critical points in the food supply chain, which is generally considered as key in enhancing food safety.

Food safety has been an increasing concern for consumers, retailers and all production and processing areas of the food industry. Food safety is also of crucial importance from economic point of view (i.e. medical costs, lost productivity and premature deaths). Although the safety of food has significantly improved overall, foodborne outbreaks from microbial contamination, chemicals and toxins are still common in many regions including highly developed countries. Indeed, the number of foodborne illnesses associated with the consumption of e.g. minimally processed produces as well as muscle-based, fully cooked and ready-to-eat food has increased.

In this context, the development and application of quality assurance systems based on prevention through monitoring of food parameters throughout the whole supply chain appears to be a prerequisite. Through their advanced communication functions, intelligent packaging concepts can play an important role in ensuring food safety.

19.2 Concepts of intelligent packaging

Intelligent packaging (IP) is typically defined as small, inexpensive labels or tags that are attached onto primary or secondary packaging in order to facilitate communication throughout the supply chain. IPs can be classified as: (1) quality indicators (e.g. gas indicators, time–temperature indicators (TTIs) or freshness indicators); or (2) intelligent packaging which provides more convenience, improved traceability or improved logistical handling (e.g. radio frequency identifications (RFIDs), microwave doneness indicators or thermochromic inks).

Based on an alternative classification IPs can also be divided into sensors and indicators; some overlap is unavoidable, however. Most sensors contain two basic functional units: a receptor and a transducer. In the receptor, physical or chemical information is transformed into a form of energy, which may be measured by the transducer. Unlike sensors, indicators do not comprise receptor and transducer components and communicate information through direct visual change (Kerry *et al.*, 2006).

Some of the IPs actually monitor the storage environment rather than the product itself or the headspace in package (e.g. TTIs, RFIDs), and therefore offer predictive, indirect indications of food quality based on established correlation. Others directly monitor the metabolites of biochemical and microbial activity that bring about changes in freshness and safety (Sharrock, 2012). Among various IP concepts, TTIs and RFIDs are by far the most adopted technologies in the market.

19.2.1 Time-temperature indicators

The basic idea behind temperature-related food quality indicators is that the quality of food generally deteriorates more rapidly at higher temperature due to an increased rate of microbial proliferation, metabolic activities and other chemical-, sensorial- and nutritional reactions. Maintaining correct storage temperatures is therefore a requisite for ensuring food safety and quality. However, conditions during transportation and at the retail level are out of manufacturer's direct control and, as generally recognized by manufacturers, retailers and food authorities, storage temperatures often deviate from specifications (Tsironi *et al.*, 2008).

Temperature should be monitored throughout the whole food chain from production via distribution and storage including domestic storage at the consumer level (Nuin *et al.*, 2008).

As temperature data loggers are relatively expensive and their use is time-consuming, alternatives to monitoring temperature are required. Time–temperature indicators (TTIs) are defined as cost-effective user-friendly devices that provide a visual summary of a product's accumulated chill-chain history, recording the effects of temperature in time at product unit level (Taoukis and Labuza, 1989). Their function is based on mechanical-, chemical-, electrochemical-, enzymatic- or microbiological change, which is often communicated as a visible signal in the form of a mechanical deformation, colour development or colour movement (Vaikousi *et al.* 2008).

TTIs may be classified as either partial-history or full-history indicators, depending on their response mechanism. Partial-history indicators only respond when a temperature threshold has been exceeded, while full-history TTIs give a continuous temperature-dependent response throughout the history of a product. Depending on the target application, one or the other could be selected. For instance, full-history indicators are able to monitor the temperature throughout the supply chain from the manufacturer to the consumer. On the other hand, partial-history TTIs can support consumers when deciding whether a food product is safe to consume or not.

Ideally, a TTI should be selected so that the activation energy E_a of the reaction taking place within the device matches with that of the microbial, chemical and/or biochemical reaction responsible for quality deterioration or spoilage in the product. When the food and the device become exposed to the same temperature–time history, the extent of reaction that occurred in the TTI can be related to the extent of reaction that occurred in the product and the integral effect of temperature can quantitatively be translated to food quality. Critical prerequisites for the valid application of this approach are the systematic study and kinetic modelling of the temperature-dependent reactions that establish

shelf-life criteria of the product as well as the response kinetics of the TTI (Simpson *et al.*, 2012).

Further requirements of an effective TTI include robustness, non-toxicity, small size, low cost and long pre- and post-activation shelf-life. TTIs should also be unaffected by environmental conditions other than temperature (i.e. light and humidity), be easily integrated into the food packaging and convey information in a clear manner (Hogan and Kerry, 2008; Vaikousi *et al.*, 2008).

19.2.2 Current technologies and applications

TTIs currently commercially available on the market can be categorized into molecular diffusion, enzymatic, polymer-based, solid-state reaction and microbiological systems. Some of the major devices along with their principle of operation are briefly described in the following.

A diffusion-based TTI, Monitor Mark® (3M Company) relies on the temperature-dependent diffusion reaction of a coloured fatty acid ester along a porous wick made of high-quality blotting paper. Its measurable response is the distance of the advancing diffusion front from the origin (Kerry *et al.*, 2006). Freshness Check®, another product from the same supplier, uses a viscoelastic material that migrates into a diffusively light reflective porous matrix, progressively causing a visual temperature-dependent increase in the light transmission of the porous material (Vaikousi *et al.*, 2008).

TT Sensor™ (Avery Dennison) consists of an indicator label and a transparent activator overlay, which can be brought together by a special dual-spindle applicator only when activation is needed. This allows the indicator to be stored in an inactive form for up to six months. Once activation has taken place, diffusion of an acidic species from one polymer layer to the other results in a progressive, irreversible colour change from fluorescent yellow to bright pink at a rate dependent on time and temperature (Sharrock, 2012). No refrigeration of the labels prior to application is required.

As an example of enzymatic systems, Vitsab's Check Point® I is based on a colour change in the TTI induced by a pH drop resulting from the controlled enzymatic hydrolysis of a lipid substrate, which changes the colour of the chromatic indicator from green over yellow to orange-red (Tsironi *et al.* 2008). Activation of the TTI is done by mechanical breakage of a seal separating the enzymes and the substrate. Vitsab has also developed Check Point® III, which is a diffusion-based TTI exhibiting rapid response at high, abusive temperatures.

Fresh Check® and Freshness Monitor® TTIs (Lifelines) use temperature-dependent polymerization reactions in which disubstituted diacetylene crystals polymerize into a highly coloured polymer (Nuin *et al.*, 2008; Vaikousi *et al.*, 2008).

The OnVu™ (Ciba Specialty Chemicals) is an example of a solid-state TTI system, which is based on photosensitive compounds such as benzylpyridines. Upon activation, these are excited by exposure to UV light causing a blue colour of the indicator which then turns back to the initial colourless state in a time–temperature-dependent spontaneous solid-state crystal phase reaction. By controlling the type of the photochromic compound and the length of light exposure during activation, the temperature sensitivity of the TTI can be controlled. This TTI can also be printed directly onto the packaging in a cost-effective manner.

TRACEO® and (eO)® developed by Cryolog are microbiological TTIs consisting of selected strains of lactic acid bacteria. Prior to utilization, these TTIs are stored in a frozen state (−18 °C) to prevent bacterial growth in the TTI medium, while their activation is obtained simply by defrosting them. (eO)® is an adhesive label in the form of a small gel pad shaped like the petals of a flower that changes from green to red. Such irreversible colour transformation indicates a pH drop due to microbial growth of TTI microorganisms within the gel itself (Ellouze and Augustin, 2010). TRACEO® is a transparent adhesive label placed over the barcode of refrigerated products, which becomes red when the product has been subject to a critical accumulation of ruptures in the cold chain and thus makes

reading of the barcode impossible. In principal, such microbial TTIs are suitable for monitoring the shelf-life of food products that are spoiled primarily by lactic acid bacteria or other bacteria exhibiting similar kinetic responses and spoilage potentials (Vaikousi *et al.*, 2009).

19.2.3   State-of-the-art developments

Driven by significant interest from industry, there has been a continuous research activity aiming for the development of even better TTIs. As an example of such efforts, Japanese authors suggested that certain oil/water emulsions or aqueous solutions of some amide compounds can be used as simple partial TTIs, as a phase separation occurring at a defined temperature causes clear visual change (Mizoguch *et al.*, 2009). The development of a new enzyme-type TTI based on the reaction between amylase and starch has also been reported (Yan *et al.*, 2008). It was also demonstrated in this study that by modifying the proportion of amylase and starch the activation energy of the TTI can be tailored for food of different shelf-lives.

Kreyenschmidt *et al.* (2010) characterized a novel TTI based on a photochromic solid-state reaction under specific temperature conditions and UV irradiation and found such a TTI to be a reliable tool to monitor the cold chains of a broad range of food products. Another TTI prototype based on the vapour diffusion of lactic acid has recently been developed by Wanihsuksombat *et al.* (2010).

A microbial TTI prototype based on the growth and metabolic activity of a *Lactobacillus sakei* strain was proposed by Vaikousi *et al.* (2008). In such a TTI, an irreversible colour change of a chemical chromatic indicator (from red to yellow) is generated due to the pH decline that results from microbial metabolism in the indicator itself.

More recently, the intrinsic instability of Ag nanoplates was used to develop a novel class of colorimetric TTI indicators. Such a concept is based on the fact that the Ag nanoplate displays a strong, in-plane dipole resonance mode in the

visible region, whose peak position has been found to be highly sensitive to the sharpness of the corners. As the corners of the nanoplate become increasingly rounded, a gradual but significant blue shift for the resonance peak position is observed. The rate of shift (nm per day) has a strong correlation with the temperature, which could be particularly useful for accurate and sensitive recording of the time–temperature history of a product (Zeng *et al.* 2010).

A colorimetric TTI based on a fadable ink was presented by Galagan and Su (2008). This approach exploits the fact that the fading time of an anthraquinone derivative depends on the oxygen diffusion rate through a polyacrylate protective layer of ink as well as the temperature, and could therefore be used to demonstrate the time–temperature history of a food product.

An intelligent and biodegradable temperature indicator packaging material (i.e. chitosan-coated paper) has been developed based on a natural and heat-sensitive pigment (anthocyanin or ATH). The colour of the indicator changes irreversibly from light violet to light yellow in response to different temperature exposition in the range of 40–70 °C (Maciel *et al.* 2012). Despite its obvious benefits (i.e. biodegradability, easy processing) such an indicator is however not suitable for monitoring the cold chain.

19.2.4 Possibilities and limitations

TTIs have typically gained a commercial foothold on commodities for which cold chain compliance is critical for safety, for instance fish, poultry and meat. A number of validation studies have also been undertaken in order to justify the effectiveness of TTIs for different refrigerated products, including fish and seafood products (Nuin *et al.*, 2008; Vaikousi *et al.*, 2008; Ellouze *et al.*, 2011; Tsironi *et al.*, 2011; Simpson *et al.*, 2012), mushrooms (Bobelyn *et al.*, 2006) and meat products (Ellouze and Augustin, 2010).

It is worth pointing out that while TTIs are typically used to reveal whether the product has been exposed to undesirably high temperatures, for most produces storage *below* their safe minimum temperature is also critical. For tropical fruits, such as mango or banana in particular, it is difficult, if not impossible, to prove that they were subject to suboptimal storage temperatures (i.e. 1–10 °C), which makes the use of TTIs rather impractical (Sharrock, 2012).

19.3 Radio frequency identification

Radio frequency identification (RFID) systems use radio waves to track items wirelessly. They are based on tags affixed to assets (cattle, containers, pallets, etc.) to transmit accurate, real-time information to a user's information system. Tags may take many forms; however labels are the most popular. Generally speaking, RFID technology is considered to be useful for anti-counterfeiting, anti-theft and, in particular, to support supply chain management. The major benefits of RFID technology in the food industry are greater speed and efficiency in stock rotation and better tracking of products throughout the chain, all resulting in improved on-shelf availability at the retail level and enhanced forecasting (Brody *et al.*, 2008).

RFID tags may be classified into two major types: (1) passive tags that have no battery and are powered by the energy supplied by the reader; and (2) active tags that have their own battery for powering the microchip's circuitry and broadcasting signals to the reader. Sometimes a third category is also referred to. Semi-passive tags, also known as semi-active tags, only use a battery to run the circuit of its microchip, but transmit the signal using the same method as that of passive tags. These tags can be read at a higher speed as compared to passive tags. These tags can also monitor and record external conditions using sensors, which are powered by a battery. Other classifications distinguish between low-, intermediate- and high-frequency RFIDs. More detailed discussions on different RFID types and their mode of operation can be read elsewhere (Kumar *et al.*, 2009).

Although RFID has been available for many years for tracking expensive items and livestock, its broader application in food packaging has only begun in recent years. In principle, an RFID tag can be attached to a package which then becomes intelligent in a sense that the stored data provide valuable information that can be stored and read by appliances. Read and write operations – features of some RFID tags – allow real-time information updates throughout the supply chain.

When considering the potential of RFID technology in enhancing food safety, it may be concluded that such technology is still in its early stages of implementation; currently, the focus is on relatively simple tasks such as product identification and tracking and not on complicated matters that involve the application of scientific food principles. Indeed, the contribution of RFID technology to assure food safety has been realized so far through enhanced traceability (Mai *et al.*, 2010).

On the other hand, RFID tags may also be integrated with a temperature logger, as well as with a chemical sensor or a biosensor to carry time–temperature history, chemical and microbial data, thus enhancing food safety in a more explicit manner. For example, a recent paper by Shi *et al.* (2010) presented a real-time monitoring solution for cold chain distribution by integrating RFID, sensor and wireless communication technologies and highlighted the advantages from the manufacturers' and retailers' perspective. In fact, RFID tags which are able to sense and integrate temperature over time are already available on the market.

RFID technology has also been used in monitoring the ripening of climacteric fruits during transportation and vending. As an example, Vergara *et al.* (2007) developed a prototype of an RFID system with metal oxide sensors on board the reader for monitoring the ripening of apples. Other RFID-based sensors for monitoring chemical analytes have also been reported. However, typical drawbacks of such sensors are difficulties in accurate measurements in the presence of interferences, difficulties in quantitation of multiple parameters with a single sensor and the need for costly development of dedicated transducers for sensing. Potyrailo and Morris (2007) claimed to address all these issues and demonstrated that a multianalyte chemical identification and quantitation can be achieved by applying a chemically sensing film onto a conventional RFID tag, measuring complex impedance response from the RFID sensor and applying multivariate statistical analysis methods to extract analyte-related information from the complex impedance response. As industry-standard passive RFID tags are relatively cheap, such an application could also be economically viable. Apparently, cost issues are among the main challenges in implementation of RFID technology in general.

Recent advances in printed electronics technology have however opened up the possibility of producing cheap, wireless sensor systems as an alternative to RFIDs currently based on silicone semiconductors. Printed conductor structures can be produced, for example, using conductive silver nanoparticles by electrical sintering (Allen *et al.*, 2011a, b). This approach has been used to produce a prototype of a wirelessly readable spoilage sensor for poultry that is based on the reaction of silver with H_2S.

Inkjet-printed single-wall carbon nanotube (SWCNTs) patterns have also been considered as a cheap solution for wireless sensor system (Cao and Rogers, 2009). In principle, one or more of the different conductive inks might be made inherently sensitive to a target analyte in a way that affects its conductivity and thus the complex impedance of the pattern when interrogated by a wireless reader (Sharrock, 2012).

Although the unit price of an RFID tag seems to have decreased significantly, the infrastructure required for RFID systems (including readers, database servers with communication systems and other information technology) still remains costly and needs to be taken into account (Brody *et al.*, 2008).

Besides cost there are some technical issues related to, for instance, reliability and readability, as well as recycling difficulties; these issues remain challenging in the implementation of

RFID technology in general. Consumer concern about privacy and security is also a hindrance in public acceptance of RFIDs (Kumar *et al.*, 2009).

Looking at the applicability of RFID tags from the consumer's perspective it should be emphasized that all the applications described above are typically designed for monitoring the supply chain by manufacturers and retailers, and not intended for communicating quality and safety-related information directly to consumers. A broad application of RFID-based sensors specifically in individual consumer packaging items is, at least, uncertain in the near future.

For further information on the potential application of RFID technology, particularly in the food industry, the interested reader is referred to recent publications (Kumar *et al.*, 2009; Mai *et al.*, 2010; Raab *et al.*, 2011; Harrop, 2012).

19.4   Gas indicators and sensors

Modified atmosphere packaging (MAP) is a typical example of how the packaging industry has addressed the increased consumer demand for fresh, minimally processed/mildly preserved, refrigerated food products with prolonged shelf-life. Package integrity for MAP is of paramount importance for ensuring the safety and quality of packaged food. Using low oxygen (O_2) and relatively high carbon dioxide (CO_2) concentrations in MAP facilitates extension of product shelf-life, improvement in appearance, reduction in food deterioration due to lipid-oxidation, impeded microbial growth and other factors. Monitoring the actual concentration of such gases in the package headspace by means of gas indicators or sensors therefore seems essential.

In principle, gas *indicators* in the form of a package label or printed on packaging films which signal changes in gas composition, could be used as a leakage indicator or even to verify the efficiency of, for instance, an oxygen scavenger.

The concept of optical on-pack *sensors* for monitoring the gas composition (i.e. O_2 and CO_2) of the MAP package at different stages of the

distribution process is a very attractive alternative to the conventional destructive gas monitoring techniques such as gas-chromatography (GC). Indeed, there has been intensive research activity aimed at developing an ideal gas indicator or sensor which could then contribute to quality assurance in MAP and also lead to greater consumer confidence in the integrity of food packaging (Mills, 2005). Some of the main developments are presented briefly in the following sections.

19.4.1   Oxygen indicators

Oxygen indicators have been proposed to visually determine the integrity of low-oxygen-concentration MAP (Smolander *et al.*, 1997). It is generally acknowledged that an ideal oxygen indicator should be inexpensive and not add significantly to the overall cost of the package. It should also not require expensive analytical instrumentation for reading its signal, but should be easily and obviously recognizable even by an untrained person (e.g. the consumer). As the indicator is generally placed inside the package, it should comprise non-toxic, non-water-soluble components that have food contact approval. The indicator should be easily incorporated into the food package, preferably in the form of printable ink. A further essential requirement is that the indicator should be inactive under ambient conditions and only be activated as an oxygen indicator when the package has been sealed under low O_2 MAP (Mills, 2005). An ideal oxygen indicator should be tuneable with respect to oxygen sensitivity, i.e. utilize a chemistry that is readily and easily modified so that the indicator can be made to respond to changes at the 0.1% level (for oxygen-scavenged packages) or at the 0.5–2% level, for non-scavenger MAP packages. Finally, an ideal oxygen indicator for the food packaging industry should also exhibit an irreversible response towards oxygen (Mills, 2005). From a food safety point of view, reversibility is undesirable since the O_2 entering the package will be consumed during microbial growth – a consequence of the loss of integrity – and the indicator colour will improperly suggest that the package is still

intact. All of these requirements are challenging to address, which probably explains the relatively limited number of commercial applications.

Among oxygen indicators colorimetric based are by far the most investigated types. In the case of such indicators a colour change is observed, in most cases due to either: (1) an oxygen-binding reaction; (2) a redox reaction; or (3) a light-activated redox reaction.

Oxygen-binding complexes that have been investigated as candidates for oxygen indicators include haemoglobin and myoglobin, as well as synthetic metal complexes such as bis(histidinato) cobalt(II). However, these types of indicators were either reversible or exhibited poor colour change and were sensitive towards changes in pH and humidity, thus rendering them far from ideal as a potential oxygen indicator for MAP (Mills, 2005).

Redox dye-based colorimetric indicators typically comprise a redox dye such as methylene blue (MB) and a strong reducing agent, such as glucose in an alkaline medium. The most prominent commercial product, the Ageless Eye™, is made of the above-listed components along with a red, redox-insensitive dye, such as Acid Red 52, and pressed to form a pellet. In the absence of oxygen the reduction of the blue-coloured MB to its colourless leuco-form (LMB) is the dominant reaction that results in a pink colour of the indicator. When oxygen is present the dominant reaction becomes the oxidation of LMB to MB, which is associated with the development of a purple colour (Lee et al., 2005).

There are, however, some negative features that appear major barriers when the general applicability of the Ageless Eye™ and other optical sensors based on such reaction chemistry is considered. Their response, for instance, is reversible and influenced by both humidity and the presence of CO_2 (i.e. a gas for MAP). They require storage under anaerobic conditions, otherwise the reducing sugar is rapidly consumed and the indicators stop working. In order to avoid leaching of the water-soluble components upon direct contact with foods with high moisture content, such indicators should be encapsulated in a clear oxygen-permeable

ion-impermeable sachet, which also makes them relatively expensive to produce (Lee et al., 2005; Mills, 2005).

A triggered oxygen indicator system, on the other hand, would circumvent the need to carefully store the reactive form prior to use. The technology of light-activated redox dye-based oxygen indicators offers many attractive features over the others described above. In particular, such indicators do not act as an oxygen indicator until activated with light and have no components that readily react with oxygen and, as a consequence, no anaerobic storage is required. Upon irradiation the indicator is bleached, provided no oxygen is present, and recovers its initial colour upon exposure to oxygen. They also exhibit irreversibility, reusability and potential production as an ink (Mills, 2005).

The major drawback of the early systems was that these could be activated by visible light that is obviously present, for instance, in most supermarket food cabinets. What is required instead is an oxygen indicator ink that can be activated with only UV light. Such systems generally consist of a semiconductor photosensitizer (SC) coupled with a redox dye (D_{ox}) and a sacrificial electron donor (SED) in the form of an intelligent ink. These are dispersed in hydroxyethyl cellulose (HEC) which acts as an encapsulation medium. In such a system, the semiconductor, usually TiO_2, absorbs UV light in an activation step, creating electron–hole pairs. The photogenerated holes react rapidly with the SED, usually glycerol, leaving the photogenerated electrons to react with the redox dye, usually methylene blue (MB), thereby generating a differently coloured (usually bleached) reduced form of the dye (Mills and Hazafy, 2008).

It has been demonstrated that the type of UV light required for activation can also be varied by using semiconductors with different band gaps (Mills and Hazafy, 2009). Unlike its TiO_2-based predecessor, a nanocrystalline SnO_2-based oxygen indicator is not activated by UV-A light from white fluorescent lamps, but by UV-B light, which provides better control in the activation of the indicator. By using other redox dyes with potentials more positive than that of MB, it is

also possible to create less-sensitive UV-activated oxygen indicators; these are required for monitoring numerous fresh-cut produce, typically packaged under $2-5\%$ O_2 levels. Roberts *et al.* (2011) recently investigated the incorporation of a set of electrochromes including, e.g. polyviologens and thionine, and demonstrated that these can be used to produce oxygen indicators with decreased sensitivity to oxygen (up to 4%). Furthermore, such indicators with different oxygen thresholds could be combined to produce a quasi-quantitative indicator that would show different colouration as the oxygen concentration increases (Roberts *et al.*, 2011). The sensitivity of a particular indicator system can also be altered by using encapsulating polymers of different oxygen permeability (Lee *et al.*, 2005).

Beside improvements regarding the performance of these light-activated O_2 indicators, some issues in connection with their processing and safety have also been addressed recently. In particular, with the development of solvent-based (i.e. waterproof) indicators, direct contact with water (i.e. food) without loss of dye, as well as direct printing on apolar polymers (e.g. PE, PP) became possible (Mills and Lawrie, 2011; Lawrie *et al.*, 2013). As well as direct printing on packaging, incorporation of O_2-sensitive pigment to form UV-activated, O_2-sensitive smart plastic film via an extrusion process has been demonstrated by Mills *et al.* (2012). Such an approach is claimed to have potential as a 'consume-within' indicator in the food packaging industry.

19.4.2   Carbon-dioxide indicators

Developments for monitoring CO_2 level in food packaging have typically been driven by two distinct demands. The correlation between CO_2 concentration and the growth of microorganisms is widely acknowledged. Generally speaking, elevated CO_2 level (in non-MAP or nitrogen MAP) can be considered as one of the primary indicators of food spoilage. Hence, detection of CO_2 gas by colorimetric indicators provides an indirect way to indicate food spoilage. Such a group of CO_2 indicators are therefore also referred to as spoilage or freshness indicators. A relevant example for this concept has recently been presented. A mixed-pH dye-based on-package colorimetric indicator for real-time monitoring of intermediate-moisture desserts was developed by Nopwinyuwong *et al.* (2010). These authors demonstrated that the colour change to carbon dioxide (CO_2) correlated well with microbial growth patterns in dessert samples packaged in non-MAP.

Relatively high CO_2 is commonly used in MAP with the aim of suppressing microbial growth. A significant decrease of CO_2 level in such MAP food could be a sign of lost package integrity (i.e. a leak indicator). A scenario such as a decrease of CO_2 due to a leak in a package followed by an increase of CO_2 level as a consequence of microbial contamination, resulting in a final concentration similar to that of initial, can easily be imagined however. Reliable indication of microbial growth for high CO_2 MAP food is therefore rather challenging (Smolander *et al.*, 1997).

Colorimetric CO_2 indicators are attractive due to their inexpensive manufacture, quick response and recovery times as well as remarkable colour changes that are typically due to the presence of a pH-sensitive dye. While solvent-based CO_2 indicators suffer from short shelf-life and require storage under inert atmosphere, their water-based counterparts were found to have increased operational lifetimes under ambient conditions (Mills and Skinner, 2010). Their response however is directly related to relative humidity and the dye is prone to be leached when in direct contact with water (or food).

As well as application of pH-sensitive dyes, alternative approaches for optical CO_2 monitoring have also been published. In a report by Han *et al.* (2010) a diamine/diol complex was utilized to undergo a reaction with CO_2 and a strong change in helical twisting power and colour in a cholesteric liquid crystals (CLC) system that can be detected by the naked eye. Such an approach may be implemented in an economical/high-volume production process, such as screen- or inkjet printing, to compete successfully with current monitoring techniques which usually require

expensive analytical instrumentation. As demonstrated by these authors, the concept can also be used for oxygen monitoring by using a responsive binaphthyl dithiol derivative. Such an oxygen monitoring process is irreversible and indicates the cumulative exposure to oxygen.

19.5 Gas composition sensors

Unlike gas indicators, respective sensor applications typically require some dedicated purpose-built instrumentation for the assessment of their response; these sensors are therefore not primarily intended for use by the customers. Instead, gas sensors can be exploited by retailers and wholesalers as non-destructive tools for monitoring package integrity and MAP during the supply chain. An insight into the application of oxygen and carbon dioxide sensors is provided here, while more detailed discussions can be obtained elsewhere (Mills, 2005; Puligundla *et al.*, 2012).

Although details vary, most optical sensors for O_2 are luminescence-based where the luminescence intensity or lifetime of the indicator is measured and usually found to decrease with increasing partial pressure of oxygen. Such sensors typically comprise a long-decay photoluminescent dye embedded in a polymer encapsulation medium (e.g. silicone rubber, silica gel, polymers, sol–gel matrices) forming solid-state sensors (spots or membranes), which can then be incorporated into food packages. Luminescent complexes of ruthenium(II), phosphorescent platinum(II)- and palladium(II) porphyrins are the main classes of oxygen-sensitive dyes, which now dominate in oxygen sensing.

Since luminescence intensity is affected by dye bleaching and leaching, temporal variations in the intensity of the excitation light intensity and variations in the responsivity of the luminescence detector, measurement of lifetime is usually preferred instead. Indeed, the major current commercial oxygen sensor system used in food packaging research, OxySense™, uses lifetime measurements to interrogate the luminescent probes called O_2xyDot™ (Mills, 2005). The probe is attached inside and illuminated with

a pulsed blue light from a LED, some of which is absorbed by the Ru complex. The resulting red emitted light is detected by a photodetector and the measured lifetime can then be related to oxygen concentration.

Dry optical sensor film was designed for CO_2 monitoring using luminescent and pH-sensitive 8-hydroxypyrene-1, 3, 6-trisulfonic acid (HPTS) dye, a phase transfer agent (tetraoctyl ammonium hydroxide, TOAH), a polymer (ethyl cellulose, EC) and a plasticizer (tributyl phosphate, TBP) (Mills and Chang, 1993). The principle of such a concept is that mixing of pH-sensitive hydrophilic indicator dye anion with phase transfer agent result in formation of ion pairs, Q^+D^-, which can be dissolved in hydrophobic solvents and subsequently cast, printed or spin-coated to form thin films (Puligundla *et al.*, 2012).

As the sensor dots are placed inside the package, leaching of any potentially hazardous compound is a major concern. A study by O'Riordan *et al.* (2005) demonstrated that levels of migration from six types of sensors, based on different oxygen-sensitive dyes and polymers, into food simulants were within the acceptable levels, and that the sensors have satisfactory stability in these conditions.

For more complete coverage of the subject the reader is directed to reviews by Mills (2005) and Puligundla *et al.* (2012).

19.6 Freshness or spoilage indicators

The terms 'freshness indicator' and 'spoilage indicator' are sometimes used interchangeably and can mean different things to different people. As a common feature, unlike for instance TTIs, these indicators directly monitor the metabolites of biochemical processes or microbial growth that bring about changes in freshness and safety. These metabolites include organic acids (e.g. L-lactic acid, D-lactate and acetic acid), volatile amines or sulphuric compounds (e.g. H_2S).

In general, freshness indicators can be divided into dosimeters (i.e. cumulative, irreversible) and current-status indicators (i.e. non-cumulative,

reversible). The latter have the potential to revert towards their original starting colour, which in certain situations (e.g. in case of lost package integrity) may mislead the consumer by giving a false sign of freshness (Sharrock, 2012).

Although the potential of freshness indicators is generally appreciated, their widespread commercial application has been in most cases rendered impractical for numerous reasons. Such indicators are typically more expensive than TTIs to manufacture and may suffer from instability during storage. Due to their basic principle of operation, they also need to be placed inside the packaging which makes them subject to increasingly strict food contact regulations as reviewed recently by Restuccia *et al.* (2010). There is also a significant reluctance of wholesalers and retailers, since a warning signal of a freshness indicator would prevent the product being sold at the best price. Apparently, they are unwilling to absorb the extra cost that a freshness sensor adds to a product line and would simply pass the cost on to the consumers instead (Sharrock, 2012).

The number of scientific publications related to package indicators for spoilage or freshness of food is relatively limited. Among the few examples, Pacquit *et al.* (2007) presented a colorimetric dye-based sensor and indicator for monitoring fish spoilage on the basis of the presence of total volatile basic nitrogen (TVB-N). However, as the colour change of the indicator triggered only when the fish was already spoiled, it cannot be used to predict the remaining shelf-life.

More recently, Heising *et al.* (2012) demonstrated a non-destructive method for monitoring headspace ammonium as an indicator for changes in the freshness status of packed fish using ion-selective electrode in an aqueous phase. The method presented could be the basis of a more sophisticated freshness sensor, and consists of a chip and minimized electrode in a gel that contains the aqueous sensor phase.

A particular area where freshness indicators could gain a market foothold is packaged fruit produce. Many of them are harvested, packed and distributed in an unripe condition in order to minimize damage upon handling and shipping. Ideally, they reach their optimal level of ripeness

(i.e. best eating quality) while on display or shortly after a consumer has purchased them. Fruit freshness indicators, often referred to as ripeness indictors or sensors, are therefore viewed more positively by all concerned including wholesalers, retailers and consumers (Sharrock, 2012). The ripeSense™ indicator, which is on the market for pears and avocados, changes progressively and irreversibly from red through orange to yellow as a cumulative response to volatiles that accumulate in the headspace of customized packaging as the fruit ripen.

Recently, a ripeness indicator prototype for apples has been reported containing molybdenum (Mo) chromophores that change under the impact of ethylene in a colour spectrum from white/light yellow to blue due to a partial reduction of Mo(VI) to Mo(V) (Lang and Hübert, 2012). The indicator is claimed to be applicable for ripeness gauge on single fruit or in paperboard crates.

Although ripeness indicators could be successful for certain fruits, as confirmed by the success of ripeSense™ for instance, they still remain a challenge to produce at a cost that will allow widespread use on commodity fruits.

19.7 Biosensors and nanosensors

There is a general consensus that there is a need for rapid, accurate, on-line sensing for *in situ* analysis of pollutants, detection and identification of pathogens and monitoring of post-processing food quality parameters (Yam, 2012). The development of biosensors has therefore gained favour in recent years not only for clinical applications but because, in principle, they are assumed capable of fulfilling the demands described above in the area of food analysis.

By definition, biosensors are compact analytical devices that are able to detect, record and transmit information related to biological reactions. They typically comprise a *bioreceptor* (e.g. enzymes, hormones, antibody/antigens, microbe, nucleic acid) specific to the target analyte and a *transducer* responsible for the conversion of biological signals to a measurable electrical response.

This latter can assume several forms (e.g. electrochemical, optical, acoustic, mass sensitive) depending on the parameters being measured (Yam, 2012).

Although many innovative platforms have been developed for the detection of pathogens and contaminants, most of these are incorporated within devices and require the extraction of a sample to determine the presence of the target analyte, making them impractical for use as biosensors in intelligent packaging. Other challenges for such systems are that ideally they should provide an easily distinguished response (i.e. colour change) and be cheap to manufacture, which are typically not characteristics of biosensors widely applied in clinical, biochemical and environmental analyses (Kuswandi *et al.*, 2011).

The convergence of nanotechnology and biotechnology has been developing for some years and is now is picking up pace; this is also true in biosensor development. Indeed, numerous research reports describe detection methods for bacteria, viruses, toxins and allergens using nanotechnology. The rationale behind these developments is that the particular optical, electrochemical or magnetic properties of nanomaterials may increase the speed and the detectability of the diagnostic methods. In particular, the small size of nanoparticles relative to those of the target organisms causes large and readily observable electrical/optical property modulations before and after binding events (Duncan, 2011). Nanosensors, by definition, are nanotechnology-enabled sensors characterized by one of the following attributes: either the size of the sensor or its sensitivity is on the nanoscale or the spatial interaction distance between the sensor and the object is in nanometres (Khanna, 2008).

Theoretically, via detection of certain chemical compounds, pathogens and toxins, nanosensors could offer real-time monitoring of food safety and freshness, provided that they could be integrated into food packaging. In the context of such effort, the use micro- and nanoelectromechanical systems (MEMS and NEMS) might also play an important role in realization.

To date, however, nanosensors usually comprise a part of equipment that is far too elaborate and expensive to be considered for food package monitoring applications. There are some examples of nanosensors technology that could however be simplified to function possibly as sensor labels on packaging (Sharrock, 2012).

Although a comprehensive discussion on bio- and nanosensors is outwith the scope of this chapter, a vast amount of relevant information can be obtained by the interested reader from excellent scientific papers and reviews published recently (Zhang *et al.*, 2009b; Velusamy *et al.*, 2010; Rebe Raz and Haasnoot, 2011; Cederquist and Kelley, 2012). Many of these publications have been written in the context of pathogen detection in particular (Sanvicens *et al.*, 2009; Velusamy *et al.*, 2010; Gehring and Tu, 2011; Gilmartin and O'Kennedy, 2012; Shinde *et al.*, 2012).

The aim here is rather to give insights into some of the major technologies which, in principle, provide potential to integrate such sensors inside or into food packaging, thus adding intelligence to the packaging. In particular, the use of metallic nanoparticles (NPs), quantum dots (QDs), carbon nanotubes (CNTs) and conducting polymers (CPs) has been investigated for their potential applications in sensors. Further, DNA-based nanostructures have also been explored as tools for molecular detection schemes and are therefore discussed briefly in Section 19.7.3. Others, for instance various novel label-free nanosensor technologies such as nano cantilevers, nano surface plasmon resonance (SPR), surface-enhanced Raman scattering (SERS) or quartz crystal microbalance (QCM), are not discussed here but can be read of elsewhere (Duncan, 2011; Gilmartin and O'Kennedy, 2012; Rodriguez-Lorenzo *et al.*, 2012).

19.7.1   Metallic nanoparticles

Due to their high surface to volume ratio, metallic nanoparticles such as gold and silver nanoparticles can be used for signal amplification, resulting in increased sensitivity of nanosensors. Gold nanoparticles (Au NPs) in particular seem to be the focus of current research and are used in a number of optical and electrochemical bioassays (Wang *et al.*, 2010a; Syed

and Bokhari, 2011; Dreaden *et al.*, 2012). Au NPs are also exploited in SPR sensor, SERS-based- or QCM-based sensing, as well as in bio-barcode assays (Saha *et al.*, 2012).

Generally speaking, various bioreceptors can be immobilized on the surface of gold nanoparticles through different coupling chemistry (e.g. thiol, dithiol, silanes, amines, carboxylic acid). The unique optical properties of Ag NPs, on the other hand, allow them to be utilized in a label-free detection approach (Cederquist and Kelley, 2012).

From a food safety perspective, detection of foodborne pathogens is of significant importance. With this aim, colorimetric assays – based on the fact that aggregated Au NPs are blue in colour while dispersed solutions of Au NPs are red – were developed recently for the monitoring of *Salmonella* Typhimurium and multiple-drug-resistant *S.* Typhimurium DT104 (Wang *et al.*, 2010b; Khan *et al.*, 2011).

A highly amplified fluorescence barcode assay based on Au NPs functionalized with a large number of oligonucleotides strands (i.e. barcodes) and a corresponding recognition agent for the rapid detection of *Salmonella* Enteritidis was reported by Zhang *et al.* (2009a). As demonstrated by these authors, a similar approach could be applied for the simultaneous multiple detection of *Bacillus anthracis* and *S.* Enteritidis (Zhang *et al.* 2010).

Further signal amplification of Au NPs can be realized using silver enhancement, as has been demonstrated recently. As detection limit as low as 5 CFU mL^{-1} for *Salmonella* sp. could be achieved in a chemiluminescence-based assay containing silver deposited around the Au NPs (Wang *et al.*, 2011). Cao *et al.* (2011) presented a similar signal amplification approach by gold and silver atoms for a simple and cost-effective optical detection of the foodborne pathogen *Campylobacter jejuni*.

The metal enhanced fluorescence effect of silver nanoparticles (Ag NPs) has also attracted growing interest for the sensitive fluorescence detection of biomolecules, including proteins. Single-stranded (ss)DNA modified Ag NPs, for instance, have been used recently for one-spot simultaneous detection of multiple proteins (Wang *et al.*, 2012).

Beside spherical NPs, gold nanorods (Au NRs) are also used for optical detection in the near-infrared. Since Au NRs have length-dependant absorptive properties, they also offer the possibility of multiplexed detection (Duncan, 2011).

Generally speaking, electrochemical detection has the advantage over optical (colorimetric or fluorimetric) methods of being unaffected by light scattering and adsorption from the various food components. With this in mind, the electrical properties of the Au NPs were exploited for the development of a piezoelectric biosensor for 'real-time' detection of *Escherichia coli* O157:H7. Briefly, target-specific (ss) DNA-functionalized Au NPs bound firstly to the target DNA and subsequently to a complementary probe immobilized onto the piezoelectric biosensor surface, resulting in frequency shift of the biosensor. Typical detection limits were reported to be around 100 CFU mL^{-1} (Chen *et al.* 2008).

In summary, metal nanoparticle-based sensing approaches have been used for detecting pathogens, proteins, metals and different small molecules, suggesting that similar strategies could be utilized for the recognition of any analytes of food safety relevance. There have been also promising experiences in the use of metal nanoparticles and SERS in terms of improvement of sensitivity and speed. For additional information on the subject, the interested reader should refer to recent comprehensive reviews (Rodriguez-Lorenzo *et al.*, 2012; Saha *et al.*, 2012)

19.7.2 Quantum dots

Quantum dots (QDs) are luminescent semiconductor nanocrystals that generally have dimensions in the range 2–10 nm and have unique electro-optical properties that fall between the molecular and bulk semiconductor regimes. QDs typically possess better brightness and photostability when compared to conventional fluorescent dyes, allowing lower detection thresholds, and they have also been proven better-suited for multicolour applications (Algar *et al.* 2010).

QDs have been used as fluorescent labels in numerous assays for the detection of some of the

most common foodborne pathogens, including *Listeria monocytogenes*, *C. jejuni*, *E. coli* O157:H7 and *S.* Typhimurium. Multiplex detection of pathogens is also possible, having detection limits comparable to those of single pathogen detection assays. This can be attributed to their broad Stokes shift that permits the simultaneous excitation of many different QDs at a single excitation wavelength (Gilmartin and O'Kennedy, 2012). Protein-based bacterial toxins, including botulinum toxin serotype A, can also be detected at picomolar (pM) levels using antibody-labelled luminescent QDs, as demonstrated by Warner *et al.* (2009).

Unlike QD applied as a label, an integrated QD is present in a system throughout a bioanalysis. While it has a role in transduction, it may also serve as a scaffold for biorecognition events. The modulation of QD luminescence as a selective response to the presence of target analyte can be achieved in several ways, including fluorescence resonance energy transfer (FRET), bioluminescence resonance energy transfer (BRET), charge transfer (CT) quenching and electro-chemiluminescence (ECL). A comprehensive overview of the basic concepts and principles underlying the use of QDs with each of these transduction methods, along with many examples of their application in biological sensing, is provided elsewhere (Algar *et al.* 2010).

Despite the apparent potential of QDs, it remains an open question whether QD-based sensors can really be utilized in intelligent packaging. Among the major challenges are the cost issue, as well as the toxicity of cadmium selenide, commonly found in QDs (Sharrock, 2012).

19.7.3   DNA-based nanosensors

Due to its high specificity, controllability and robustness, the DNA molecule can in principle be used for molecular sensing. In fact, rationally designed DNA-based nanostructures can be integrated into highly versatile, multiplexed molecular detection schemes. Typical DNA-based nanostructures for molecular sensing reported in the literature include: (1) linear DNA-mediated nanostructures with diverse nanomaterials (e.g. Au NPs, QDs, carbon nanotubes); (2) nanostructures from branched DNA such as fluorescence nanobarcodes, ABC monomers, fluorescence DNA nanotags and DNA tile arrays; and (3) functional tertiary structures of DNA. A comprehensive insight into the area of such DNA-based sensing approaches is given elsewhere (Lee *et al.*, 2010; Roh *et al.*, 2011) and only some examples are cited here.

The most straightforward use of DNA involves the ability of single-stranded DNA (ssDNA) to hybridize to complementary sequences. The hybridization event is then converted into an optical, electrical or coupled response by labelling oligonucleotides with fluorescent dyes and/or by immobilizing them onto a surface. Although these signalling mechanisms may lead to effective transduction, amplification is often needed to enhance the signal, especially when only small quantities of target are present (Lee *et al.*, 2010). Different DNA–nanoparticle conjugates have been applied in molecular detection including gold nanoparticles, QDs and carbon nanotubes (CNTs).

Branched-DNA molecules are assembled from rationally designed single-stranded oligomers and can be used for the synthesis of highly-complex DNA-based nanostructures. A DNA with branch points allows for not only multiple fluorescent labels per target, but different colour ratios that may correspond to different target sequences (Lee *et al.*, 2010). For example, Li *et al.* (2005) developed a multiplexed detection strategy based on fluorescently labelled DNA nanobarcodes. These authors demonstrated that multicolour fluorescence-intensity- encoded nanobarcodes could be fabricated by precisely controlling the dye type and number, allowing for the possibility of tens of thousands of distinct colour ratios that could each correspond to a molecular probe. Such nanobarcodes were utilized for the rapid, multiplexed detection of several different pathogenic DNA molecules simultaneously via fluorescence microscopy, dot blotting and flow cytometry with attomolar sensitivity (Stavis *et al.*, 2005).

19.7.4   Conducting polymers

Conducting polymers (CPs) such as polyaniline (PANI) and polydiacetylene (PDA) have been proposed for sensor applications. In principle, such conducting particles can be embedded into an insulating polymer matrix and changes in the resistance can be used for sensing. For example, a CP nanocomposite sensor containing carbon black and PANI was able to detect foodborne pathogens (including *Bacillus cereus*, *Vibrio parahemolyticus* and *Salmonella* spp.) based on the microorganism-specific response pattern of the sensor (Arshak *et al.* 2007). More recently, Setterington and Alocilja (2011) presented a biosensor for the detection of *E. coli* O157:H7 based on biofunctionalized electroactive polyaniline (immuno-PANI).

PDA is considered as an ideal candidate for use as a sensor due to its unique optical properties. In particular, PDA molecules can change colour from deep blue to red in response to different stimuli such as temperature, pH and the presence of biological molecules. PDA also exhibits interesting fluorescence properties. In particular, while no fluorescence is emitted by the initially polymerized blue-phase PDA, the red-phase PDA strongly fluoresces at 560 and 640 nm (Takayoshi *et al.*, 1997).

In principle, PDAs can be assembled for biological assays in two different ways: immobilized as a thin film (or Langmuir film) on a solid support or as vesicles (liposomes) in a solution. The former approach allows PDA to be incorporated into an intelligent packaging system (Pires *et al.*, 2010).

PDA films and vesicles are promising devices for the detection of bacteria and bacterial toxins. Recognition elements that bound to specific markers on the surface of bacterial cells have been typically incorporated into the PDA matrices, providing chromatic signals. It has also been demonstrated, however, that by combining a PDA film with different phospholipid bilayers that function as a biomimetic membrane platform, a colorimetric detection of bacteria even without a specific recognition element is feasible.

Such a detection scheme, presented by Scindia *et al.* (2007), is based upon bacterially secreted amphiphilic compounds that bind to the lipid/PDA film surface, thereby inducing chromatic transformations in PDA. Since the extent of such colour transformation depends both on the target strains and the phospholipid compositions, bacterial fingerprinting can be achieved through pattern recognition obtained by recording the chromatic transformations in an array of lipid/PDA films having different lipid components (Scindia *et al.*, 2007).

As another example for the use of PDA, Park *et al.* (2009) reported a PDA liposome-based biosensor for multiplex pathogen detection. Proof of concept was demonstrated using two mixtures, one containing *Cryptosporidium parvum* and *E. coli* O157 and another containing *Giardia lamblia*, *S. typhimurium* and *Encephalitozoon intestinalis* at concentrations of 10^6 CFU mL^{-1}. Within 30 min, the presence of pathogens could be detected qualitatively by the chromatic transition of PDA to red or quantitatively by the fluorescence signal (Rebe Raz and Haasnoot, 2011).

For more detailed discussion on the potential applications of PDA films as biosensors in the food industry, the reader should consult recent reviews by Pires *et al.* (2010) and Chen *et al.* (2012).

19.8   Conclusion and future outlook

It is fair to say that intelligent packaging has enormous potential for enhancing food quality and safety, in particular due its ability to communicate throughout the whole supply chain. There is intensive research activity in this area, which is continuously being supported by advances in food engineering, nanotechnology, biotechnology and information technology. The extent of research on IPs for food packaging far outweighs the commercial adoption of these concepts, however. Indeed, the number of commercial applications is relatively limited, and most of these cannot survive in the competitive and challenging

market for more than a few years. The main challenges include achieving adequate reliability, cost effectiveness and compliance with increasingly restrictive food contact safety regulations.

Apparently a group of IPs, namely those which actually monitor the storage environment rather than the product itself, seem to have a broader application potential and thus a higher chance of being commercially successful. TTIs and RFID technology, for instance, could be powerful tools to support supply chain management along with quality assurance and, as a consequence, improve food quality and safety. Through their track and trace function, RFIDs are for instance able to significantly enhance the speed and effectiveness of a product recall due to bioterrorism or infection. RFIDs combined with a biosensor or a TTI can also provide real-time information for the user and are likely to be applied as inventory management tool.

Although the food industry is a late adopter of RFID technology, the impressive cost reduction of RFID tags attributed to, for example, advances in printed electronics replacing traditional silicon chips, may facilitate the broader application of such technology in this domain.

Generally speaking, the development of direct food quality indicators and sensors that monitor food freshness and safety based on direct evidence is rather challenging and typically requires more complex knowledge of food properties as well as their deterioration processes. The future of such food quality indicators and sensors mainly hinges upon whether they can appeal to every member of the supply chain including both retailers and consumers.

Retailers are reluctant, for instance, to embrace freshness indicators that guide consumers to buying food products of the best quality and which are able to indicate if the product is of less quality and safety. It also remains a concern whether consumers will be ready to pay the extra costs of such quality indicators.

Biosensor and nanosensors to date are typically utilized in clinical and environmental diagnostics; however, it is acknowledged that there may be future opportunities to exploit them for food packaging and in particular as intelligent labels. As demonstrated in many reports, nanotechnology-based sensing of foodborne pathogens and toxins, as well as other food-safety-relevant analytes, is feasible in principle. To date these methods are better suited for supply chain quality assurance; however, printed nanosensor labels may also be available one day, intended to be interpreted by the consumer.

With obvious advances in the development of bio/nanosensors and nanotechnology-based packaging, the emergence of truly interactive, 'release on demand' packaging systems can also be anticipated in the future. By combining the benefits of active packaging with those of IPs these concepts will, for instance, be able to sense critical analytes (e.g. microorganisms) in the food and trigger controlled release of antimicrobials as a response.

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20

Consumer Perception of Safety and Quality of Food Products Maintained under Cold Storage

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Summary

Due to changing lifestyles and demography, the demand for fresh and minimally processed chilled foodstuffs is increasing. For these products, quality and safety play vital roles which are influenced by innumerable factors. Storage temperature clearly has the highest impact on quality and safety aspects; even small fluctuations could adversely affect the foodstuffs. This chapter presents an overview of food spoilage processes and shows how food quality and safety may be controlled and maintained by chilling and freezing. The functional principles, benefits and applications of different cold storage technologies, consumers' food handling in private homes and influence on quality and safety of chilled products are all discussed in detail.

20.1   Introduction

Due to lifestyle changes, increasing incomes and demographic aspects, food consumption patterns have changed significantly over the last 30 years globally. In most industrial countries, food services such as out-of-home preparation for consumption at home or restaurants have been showing increasing popularity (OECD, 2001). In Europe, the food service industry is growing by 2–3% per year against recent retail market growth of 0.5% (Payer *et al.*, 2000). In the US, this trend is even more pronounced: food spending for consumption away from home accounted for 41% in 2010 (USDA, 2012). The driving forces

include a higher share of women employed, smaller sizes of households, higher incomes, more two-earner households and waning interest in and decreasing knowledge about food preparation. Nevertheless, food quality and safety, which can be defined as 'the totality of characteristics of food products that bear on their ability to satisfy all legal, customer and consumer requirements' (Will and Guenther, 2007), play an increasingly important role in food consumption.

Regarding consumption at home, convenience, healthfulness, taste and variety are the most important aspects (Bruhn, 2010). In general, there is a trend towards an increased consumption of fruits and vegetables, meat (especially pork and poultry) and pre-prepared or easy-to-prepare meals. The food industry has responded to demand by developing new product lines which include fruit and vegetable juices and frozen or chilled pre-cut and pre-prepared meat, vegetables and fruits (OECD, 2001; Omann *et al.*, 2009). The chilled food sector is described as one of the fastest-growing and most innovative sectors in food industry (CFA, 2013).

Due to some negative publicity in recent years, artificial preservatives or additives and other techniques applied to extend shelf life such as irradiation have been rejected by the majority of consumers. Nevertheless, there is a high demand for fresh and safe foodstuff with high nutritional and sensory quality (FMI, 2010; Sloan, 2011). These facts explain why home food storage, especially refrigeration and freezing, plays an important ongoing role. They maintain original food quality and help to maximize shelf life at the same time. As time of purchase and consumption of food is decoupled, both techniques additionally provide a high level of independence and flexibility in everyday life (Pichert, 1993; Klingshirn, 2008).

In this chapter, the emphasis is first on factors affecting quality and safety of food under cold storage and on cold storage technologies used in private homes to preserve food. Consumers' perceptions of safety and quality of chilled food, and awareness of these aspects from shopping to eating, are then highlighted.

20.2   The role of refrigeration in food quality and safety

Refrigeration is a vital technique to store perishable food products at temperatures slightly above the freezing point in order to maintain their quality and to extend their shelf life (Kurzhals, 2007). In contrast to other food preservation technologies (e.g. pasteurization, sterilization, ozone or high-pressure treatment, modified atmosphere packaging, drying, canning, irradiation, treatment with pulsed electric fields, salting, sugaring or pickling), refrigeration is able to maintain the initial physical, chemical, nutritional and sensory properties to a high extent over a certain period of time (IIR, 2009). However, refrigeration is neither able to improve the quality of a product nor stop spoilage processes completely, but it can slow them down (Heap, 2000). Food under cold storage also loses its original quality after a while, deterioration processes take place and the food becomes unfit for consumption. Deterioration or spoilage is defined as an undesirable decrease in food quality, which is not intentionally induced by human beings (Kurzhals, 2007). Different spoilage processes are described more in detail in the following sections.

20.2.1   Food spoilage processes

Food spoilage is defined as 'any process leading to the deterioration of the safety, sensory quality (taste, flavor, texture, color and appearance) or nutritional value of food' (Berk, 2008). It can be caused by microbial, biochemical, chemical and physical reactions which normally interact with each other, meaning that a change in one affects the rate of change in the others (Walker and Betts, 2000).

20.2.2   Microbial spoilage

Microbial spoilage is either caused by bacteria or by fungi (moulds and yeasts). These microorganisms spoil the food by growing and multiplying in it and by producing substances that change the appearance, taste or odour of a product.

With the exception of the exterior surfaces, healthy plant or animal tissues are not or only slightly contaminated with microorganisms. However, these sterile materials usually become contaminated from different sources (e.g. people, animals, equipment, water, air, dust, soil, hides, fleece, feathers) during harvesting or slaughtering, processing and packaging. Consequently, a variety of microorganisms can be found in food. As microorganisms undergo an exponential growth, the initial level of microbial contamination has a great impact on a product's shelf life. Under optimal conditions, some bacteria may divide every 20 minutes, which corresponds roughly to a tenfold increase per hour. For this reason, good hygiene at every point along the food chain is essential (Gill, 1979; Walker and Betts, 2000; Kraemer, 2002; Klingshirn, 2010).

There are two different kinds of microorganisms: pathogenic and spoilage. Pathogenic microorganisms are the kind that cause foodborne diseases by invading the body (e.g. *Campylobacter*, *Listeria monocytogenes*, *Salmonella*) or by producing a toxin (e.g. *Bacillus cereus*, *Clostridium botulinum*, *Staphylococcus aureus*). They can grow and multiply in food without any noticeable changes in sensory. For this reason, the absence of deleterious sensory is not a reliable indicator of food safety (Walker and Betts, 2000; Kraemer, 2002).

Spoilage microorganisms cause an unpleasant change in the sensory characteristics such as awful taste, smell or appearance. Some of them are able to produce colonies on the food (e.g. moulds) and others are responsible for the production of slimes, gases, acids or enzymes, which break down cell structures and components. The consumption of spoiled food is normally not harmful (Walker and Betts, 2000; USDA, 2010a).

The growth and the metabolic processes of these microorganisms are influenced by several factors, including: intrinsic properties (e.g. water activity, pH, nutrients, structure, natural antimicrobials); extrinsic properties (e.g. storage temperature, gas atmosphere, humidity); and processing factors (e.g. physical or chemical treatment). According to their ability to grow at various temperatures, microorganisms can be divided into four different groups (Table 20.1). With regard to food under cold storage, psychrophilic and psychrotrophic microorganisms are of most concern.

20.2.3 (Bio-) chemical spoilage

Chemical reactions in food start automatically and cause changes in appearance and flavour of a product. Deterioration of food catalysed by enzymes is referred to as enzymatic spoilage. Enzymes are specialized proteins, which are used by every living organism to drive chemical reactions in its cells. After death, enzymes remain active and are responsible for autolytic processes and transformations of other substances (e.g. enzymatic browning or ripening). Destruction of cell structures leads to a release of additional enzymes, which may cause further spoilage. Similar to microbial deterioration, enzymatic deterioration is also affected by water content, pH and temperature of a product. Although a reduction in storage temperature results in lower reaction rates of enzymes, they remain active even at temperatures of $0\,°C$ and below (Kurzhals, 2007; Labuza, 1982; Bahçeci *et al.*, 2005). Oxidative spoilage (autoxidation) occurs when ambient air reacts with oxygen-sensitive food components. For example, if exposed to oxygen, fat and fatty portions of food become rancid

Table 20.1 Temperature requirements of microorganisms (Kraemer, 2002), with kind permission

Temperature (°C)	Minimum	Optimum	Maximum
Psychrophilic	−5 to +5	12–15	15–20
Psychrotrophic	−5 to +5	25–30	30–35
Mesophile	5–15	30–40	35–47
Thermophile	40–45	55–75	60–90

because of the formation of short chain carbon compounds that have a strong taste or smell and make the food undesirable to consumers. Moreover, free radicals and peroxides produced in this process can destroy vitamins A, C and E (Labuza, 1982).

20.2.4   Physical spoilage

Physical spoilage refers to changes caused by extrinsic factors such as temperature, moisture or mechanical damages, e.g. staling of bread, moisture loss or gain or physical separation of food compounds. If the outer layer of a product is physically damaged, microbial or chemical spoilage is facilitated (Klingshirn, 2010).

20.3   Effects of temperature on food spoilage and quality

20.3.1   Temperature dependency of chemical spoilage processes

The rate of chemical reactions is directly influenced by temperature. A reduction in temperature will slow down these processes. Regarding chemical reactions, the so-called Q_{10} value is widely used to describe their temperature dependency. It is defined as the factor by which a reaction rate changes for each 10 K change in temperature (Toledo, 2007). In general, it can be assumed that the rate of reaction is halved for every 10 K decrease of temperature (Brown and Hall, 2000). Because of the temperature dependence of the Q_{10} value, it should not be used over a wide range of temperatures (Toledo, 2007):

$$k_2 = k_1 \left(Q_{10} \right)^{\frac{T_2 - T_1}{10}} \qquad (20.1)$$

where k_1 and k_2 are the rates of reaction at temperatures T_1 and T_2.

The Arrhenius model (Equation 20.2) is often used to predict chemical spoilage of food during storage. Several studies have shown that the Arrhenius model agrees with losses of vitamins quite closely and over a wide range of temperatures.

However, discontinuities might occur due to phase transitions (Berk, 2008; Sun, 2012):

$$k = A e^{-\frac{E}{RT}} \qquad (20.2)$$

where k is the rate of reaction; E is activation energy ($J \, mol^{-1}$); T is absolute temperature (K); R is the gas constant; and A is a frequency factor, often taken as constant across small temperature ranges.

20.3.2   Temperature dependency of enzymatic spoilage processes

The relationship between temperature and enzyme activity can be described by a bell-shaped curve (Figure 20.1). Each enzyme has its own characteristic temperature optimum where the highest activity is reached. At high temperatures (ordinarily 50 °C and above), enzymes are destroyed completely. Low temperatures, however, merely cause a depression of their activity, which is only maintained as long as the temperature is low. If temperature rises once more, the activity of the enzyme will also rise. Even at temperatures well below the freezing point, enzymes remain active. For this reason, it is recommended that enzymes are inactivated (particularly in vegetables) by blanching prior to freezing (Berk, 2008).
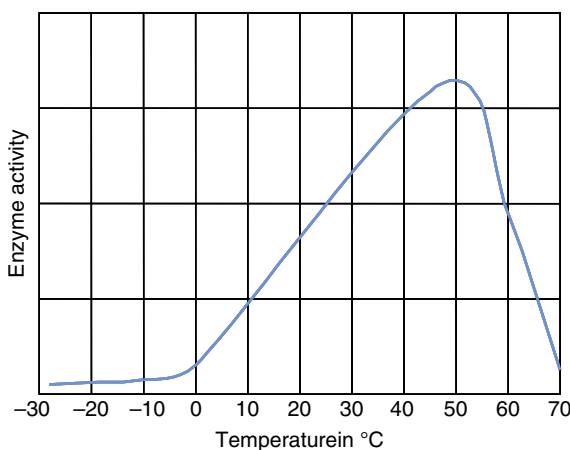
Figure 20.1   Relationship between temperature and activity of an enzyme. Source: HEA, 2009. Reproduced with permission of HEA.

20.3.3 Temperature dependency of microbial spoilage processes

Temperature is the most important factor influencing the growth of microorganisms in food. Microorganisms are divided into four different groups according to their temperature requirements for growth (Table 20.1). As shown in Figure 20.2, the growth rate of psychrophilic and psychrotrophic microorganisms are much
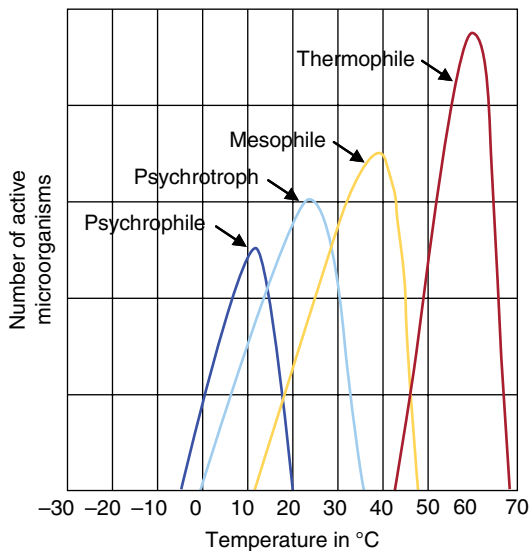
Figure 20.2 Growth rate of different groups of microorganisms in response to storage temperature (source: HEA, 2009 with kind permission) For color details, see color plates section.

lower than that of mesophiles and thermophiles, even at their temperature optimum (Stokes, 1968). At temperatures below the freezing point, microorganisms enter a dormant stage. An exponential relationship between temperature and growth rate has been reported by several authors (Willocx, 1993; Kroekel and Hechelmann, 1999; Raab et al., 2008; Bruckner, 2010). The growth curve normally appears as an S-shaped (sigmoid) curve, which has four distinct phases (Figure 20.3): an initial or lag-phase, a logarithmic or exponential phase, a stationary phase and a death phase. It is known that the lag phase, in which microorganisms adapt themselves to growth conditions, is prolonged at lower temperatures. Additionally, the growth rate during the logarithmic phase is slowed down at low temperatures, resulting in a decreased microbial load after a certain storage period (Berk, 2008).

In practise, fresh foodstuffs are seldom stored at constant low temperatures. As a result of globalization, perishable products are often transported over far distances and the transport chain comprises several steps. Within this transport chain, temperature fluctuations are quite common, especially during transition from one step to another (Raab et al., 2008; Koutsoumanis et al., 2010). Temperature measurements inside refrigerated transport vehicles indicated fluctuation within the range −5 °C and +15 °C. Surface temperatures fluctuated between 2.5 °C and 4.5 °C, whereas the temperature remains constant over a
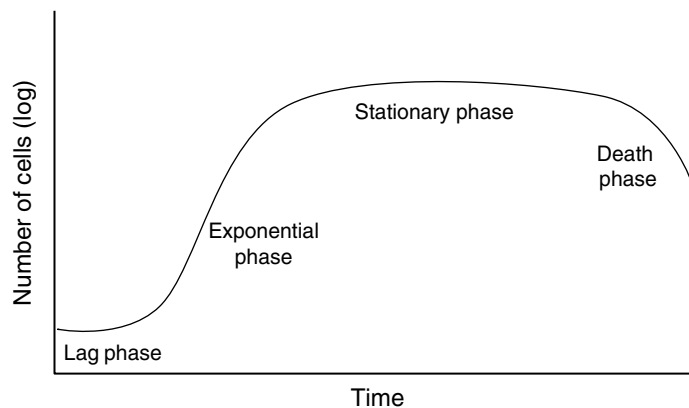
Figure 20.3 Growth curve of microorganisms and different phases

period of several minutes up to a few hours. Such temperature fluctuations can also be observed in household refrigerators (Raab *et al.*, 2008; Koutsoumanis *et al.*, 2010). Based on storage experiments with pork and poultry meat, it was demonstrated that temperature fluctuations (between 4 °C and 7 °C or 4 °C and 15 °C) may reduce shelf life by more than two days as compared to storage at a constant temperature (4 °C). When temperature shifts took place at the beginning of the storage period, shelf life reductions were significantly higher. The same applies for the total number of temperature shifts: reductions in shelf life for pork and poultry meat were more pronounced with the greater number of temperature shifts which occurred, even if the overall period of time at an abusive temperature was the same (Bruckner, 2010). This underlines the importance of maintaining the cold chain and avoiding even small interruptions.

20.4   Quality and safety of frozen foods

Freezing is one of the oldest and most widespread methods of long-term food preservation at temperatures well below 0 °C. It is able to maintain taste, texture and nutritional value of food better than any other method. At freezing temperatures, chemical and cellular metabolic reactions are reduced and microorganisms enter a dormant stage (Delgado and Sun, 2000). Chilling and freezing are not only different in terms of temperature. The preservative effect of freezing is mainly due to transition of phase from liquid to solid, which results in a reduction of molecular movement and water activity (Berk, 2008).

20.4.1   Freezing process

At the beginning of the freezing process, the material to be frozen has to cool down to a specific temperature at which a seed is formed (nucleation). This seed is required in order to start ice crystal growth and the phase change from liquid to solid. With regard to pure water, this process takes place at 0 °C. If foodstuffs are considered, the process becomes more complex as food products, besides water, may consist of salt and further soluble substances such as carbohydrates and proteins. These soluble solids cause a decrease in the freezing point of water. As a consequence, the first ice crystal in food products often appears between –0.5 °C and –5 °C. The freezing point of common fruits and vegetables is in the range of –0.8 °C to –2.8 °C. In general, the higher the molar concentration of the solutes, the lower the freezing point (Fennema, 1973; Barbosa-Cánovas *et al.*, 2005; HEA, 2009; Roos, 2012). At the beginning of the freezing process, intercellular water, being less concentrated, is converted to ice first. As a consequence, the concentration of soluble substances increases in the unfrozen water, resulting in a concentration gradient between the intercellular space and the cells. This concentration gradient is equalized by diffusion of water from the cells to the intercellular spaces. This process dehydrates the cells and should therefore be avoided as effectively as possible (Berk, 2008; HEA, 2009). For this reason, a high freezing capacity is essential to maintain the quality of frozen food (Ramaswamy and Tung, 1984). If the freezing process is slow, ice crystals will grow slowly resulting in larger ice crystals with sharp edges, which cause mechanical damage to the cell walls and an expansion of the intercellular spaces (Rahman, 1999). Rapid freezing, by contrast, avoids the formation of large, disruptive ice crystals. So, almost all of the water lost to the intercellular space can be reabsorbed into the cells during thawing and the texture is maintained to a large extent. However, food which has been frozen slowly, loses 'drip' water in the case of meat and juice in the case of fruits during thawing. An accelerated freezing rate is therefore advantageous for freezing of many food products (Barbosa-Cánovas *et al.*, 2005; HEA, 2009; USDA, 2010b).

20.4.2   Frozen storage

Is it not only the freezing process which is important in maintaining the high quality of frozen foodstuff, but also the frozen storage. Although the rate of reactions is in general lower in frozen

food, deterioration processes are not halted completely. Among the most important reactions taking part in frozen products are lipid oxidation, protein denaturation and oxidative changes such as loss in vitamins (Berk, 2008). In frozen produce, vitamins C and B are mainly affected by destruction. Concerning ascorbic acid, lower temperatures result in higher retention. At −25 °C, 10% of initial vitamin C content is lost whereas the loss is 90% at −12 °C. With the exception of folic acid, vitamin B is less sensitive to frozen storage (Klingshirn, 2010).

Moisture migration and recrystallization caused by fluctuations in storage temperature may be a major reason of quality degradation in frozen food (Berk, 2008). Temperature fluctuations may also be responsible for colour changes in frozen foodstuff. A simple experiment with green beans showed that even small temperature shifts (−18 °C to −15 °C) for a short time cause perceptible changes in colour of thawed product after a storage period of three months (Reid, 1998). Thus, maintaining constant temperatures is particularly important.

During frozen storage, small ice crystals may melt due to a lower melting point and the water may solidify on the larger crystals. This is the reason why the number of small ice crystals decreases while the size of large ice crystals increases (Blanshard and Franks, 1987).

Another frequent cause of quality loss in frozen food is so-called freezer burn. This is caused by air reaching the surface of the food and appears as greyish-brown leathery spots. Freeze burn does not make the food unsafe, it merely dries out parts of the product and causes changes in colour, texture, taste and vitamin content. Food with high water contents such as fruits and vegetables, meat and fish are particularly at risk of freeze burn. The remedy is avoiding improper, non-airtight packaging and temperature fluctuations (Klingshirn, 2010). Food safety of frozen food largely depends on the microbial state of the raw product at the time of freezing and the thawing process in consumers' homes. Freezing inactivates microorganisms and so prevents their growth. Once thawed, they may become active again and may cause the food to spoil. For this reason, it is recommended to defrost food slowly in the refrigerator (USDA, 2010b).

20.5 Cold storage technologies

In developed countries, cooling appliances such as refrigerators and freezers are commonplace in every household. According to Billiard (2002), there are about 1 billion refrigerators and freezers worldwide. With a saturation of 106%, the European market of refrigerators is already oversaturated, meaning that there is more than one appliance in some households (Stamminger, 2001). Market penetration in the US and Japan is similar. Refrigerators are used to store perishable food products at temperatures slightly above the freezing point in order to maintain their quality and to extend their shelf life (Kurzhals, 2007). For this purpose, the temperature in the storage area has to be kept at a low level by removing the heat, which enters the compartment by warm food or by conduction and convection through the cabinet walls (Whitman *et al.*, 2005).

20.5.1 Principles of refrigeration

The vapour-compression refrigeration cycle is the most commonly used refrigeration cycle in domestic appliances. The purpose of this cycle is to transfer heat from a low-temperature region (inside the refrigerator) to a high-temperature region (outside the compartment). The heat transfer is enabled by a refrigerant (e.g. saturated hydrocarbons such as isobutane) that changes its state of aggregation. This refrigerant circulates inside a closed loop piping system. A refrigeration system consists of four interlinked components: an evaporator, a compressor, a condenser and an expansion (or throttle) valve (Figure 20.4).

The liquid refrigerant is evaporated at low pressure by absorbing heat from the cooling area inside the refrigerator. After that, it enters the compressor at its boiling point. The vapour is compressed to a superheated vapour before it
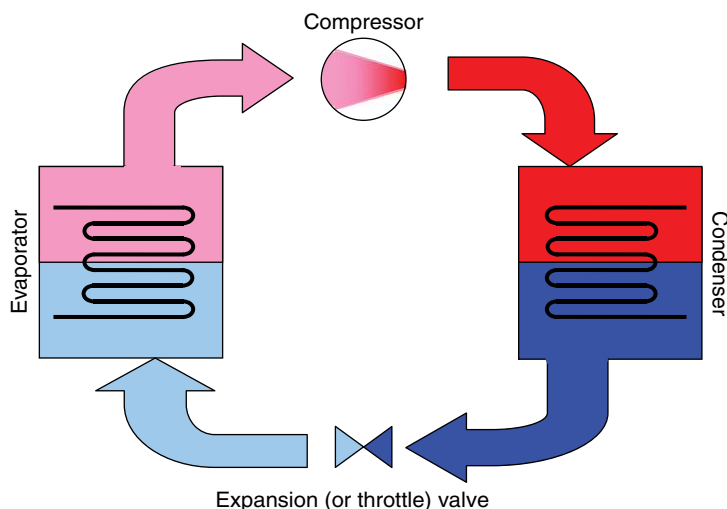
Figure 20.4   Principle and components of a vapour-compression refrigeration set. For color details, see color plates section.

leaves the compressor. In the subsequent condenser, the refrigerant rejects most of its energy to the environment and turns to a liquid state. Finally, the pressure of the refrigerant is decreased by passing an expansion valve and the cycle is completed by re-entering the evaporator (Heap, 2000; Pichert, 2001; HEA, 2009).

20.5.2 Refrigerator layout and temperature zones

The temperature inside a refrigerator or freezer is controlled by a thermostat, which consists of a temperature probe and a control knob. The latter allows users to adjust the temperature setting. In simple cooling appliances, an electromechanical thermostat is located at the evaporator, where the actual temperature is acquired. In modern appliances, the actual temperature is measured directly inside the cooling compartment using electronic temperature controllers. Actual temperature is compared with the adjusted value. If the differences exceed a predefined limit, the thermostat prompts the compressor to turn on. After cooling down to a specific designated temperature, the compressor is deactivated in the same way (HEA, 2009).

Temperature distribution inside the compartment is influenced by innumerable factors such as type of refrigerator, ambient temperature, loading with products and door openings (Klingshirn, 2008). James and Evans (1992) showed that temperature distribution within an empty storage compartment varies substantially between different types of appliances. Furthermore, temperature distribution inside a storage compartment may change as a result of loading with products. As a rule of thumb, it can be assumed that the coldest areas are at the rear wall and the lower shelves as the airflow is determined by natural convection (Figure 20.5). In some cases, the airflow rate is increased by an additional fan resulting in a more uniform temperature distribution. Temperature distribution inside a frost-free appliance is normally even more homogeneous (Figure 20.6). Here, the air is conducted by a ventilator to the evaporator, where the moisture condenses as frost. The cold and dry air is now led through an air duct at the rear wall with ventilation slots on each shelf back to the refrigerator compartment (Klingshirn, 2008).

Some modern appliances are divided into different temperature zones in order to provide optimal storage conditions for different types
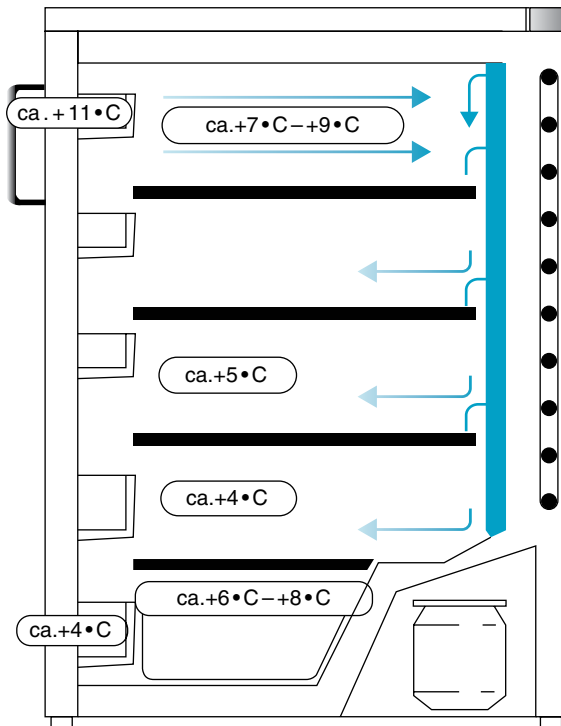
Figure 20.5   Temperature distribution inside refrigerator compartment caused by natural convection. Source: HEA, 2009. Reproduced with permission of HEA.

Cooling mode

Figure 20.6   Air flow inside frost-free refrigerator. Source: HEA, 2009. Reproduced with permission of HEA.

of food (ISO, 2005): fresh food storage compartment (for the storage of fresh, unfrozen food); chill compartment (for the storage of highly perishable foodstuffs); cellar compartment (for the storage of semi-perishable food and beverages at temperatures higher than that in the fresh food compartment); freezer compartment (for the storage of frozen foodstuffs). European freezers and refrigerators with a freezer compartment are graded with a four star rating system (ISO, 2005; Table 20.2).

In some appliances, the chill compartment additionally has a humidity control feature. Usually, it comes with two different settings: the area of low humidity (about 50% RH) is suitable for storing packed animal products such as meat and fish. Plant products, which are not packed, can be stored in the area of high humidity (about 90%). Humidity control is either realized by slide

control or by a special fleece layer integrated in the compartment cover (HEA, 2009).

20.5.3   Energy label and its influence on cooling performance

Refrigerators and freezers are appliances intended for the storage of perishable food products at temperatures slightly above (refrigerators) or well below the freezing point (freezers) in order to maintain their quality and to extend their shelf life. At the same time, they are major users of electrical energy in private homes, consuming 13.4% of total electricity in the residential sector (IEA, 2003). For this reason, several countries worldwide implemented programmes to regulate this issue. These programmes can be either introduced in the form of minimum energy efficiency standards (MEPS) or in form of comparative labels. MEPS are mostly mandatory and can be found in Australia, Europe, US and in parts of Asia and Latin America, among other countries. They are defined as 'procedures and

Table 20.2   Star rating of freezers (ISO 2005; HEA, 2009).

Star rating	Temperature range	Storage application
*-compartment	≤ −6 °C	Short-term storage of frozen foods
**-compartment	≤ −12 °C	Short-term storage of frozen foods
***-compartment	≤ −18 °C	Storage of frozen foods
****-compartment	≤ −18 °C	Storage of frozen food, freezing foodstuffs from ambient temperature down to −18 °C
Ice-making compartment	≤ −18 °C	Freezing and storage of ice

regulations that prescribe the energy performance of manufactured products, sometimes prohibiting the sale of products that are less efficient than a minimum level' (Mahlia *et al.*, 2003, 2004; Wiel and McMahon, 2005; Harrington, 2009).

Complimenting MEPS, some countries also introduced energy efficiency labels that provide necessary information allowing consumers to make a well-informed purchase decision. These labels are often attached to electric appliances or their packaging in the form of stickers. Two distinct types of labels are of particular importance: (1) endorsement labels, which indicate that a product meets or exceeds pre-specified criteria or levels (e.g. blue angle in Germany); it is not possible to identify the most efficient product among those endorsed; and (2) comparative labels, allowing comparison of performance between all available models (e.g. European Energy Label), which uses either a discrete ranking system or a continuous scale.

In order to ensure consistency and comparability, all labels and energy efficiency standards are based on specific agreed-upon methods of measuring energy performance of appliances (test procedures) (Meier and Hill, 1997; Mahlia *et al.*, 2002; Saidur *et al.*, 2005; Wiel and McMahon, 2005; Mahlia and Saidur, 2010).

The positive impact of energy labelling on energy efficiency has been highlighted by several authors (Harrington and Wilkenfeld, 1997; Waide, 2001). Caused by the implementation of the Energy Label in combination with MEPS in Europe, for example, the share of efficient class

A and B appliances in the European market for example increased from 10% in 1990–1992 to roughly 57% in 1999 (Waide, 2001). This trend also continued in subsequent years.

In view of refrigerators and freezers, major improvements in efficiency have been achieved by using better insulation materials such as vacuum panels or by using lower-dimensioned compressors. However, it could be noted that especially highly efficient appliances have long cycling times and are not particularly sensitive to a rise in internal temperatures. Such appliances might be unable to cope with the complete heat load, caused by frequent door openings or by warm foodstuffs, within a reasonable period of time even though they are operating continuously (Geppert, 2011). As a consequence, energy savings may only proceed as far as food quality and safety as well as nutritional aspects and health are not compromised.

20.6   Consumers' handling of chilled food and home practices

Worldwide, cases of foodborne diseases have been dramatically on the rise in recent decades (WHO, 2012). For industrialized countries, it has been reported that up to 30% of the population have suffered from some kind of foodborne illnesses (WHO, 2002). About half of all outbreaks are caused by improper food handling at home, including inadequate refrigeration and (re-)heating as well as cross-contaminations

(WHO, 2000; Scott, 2003). According to Evans (1998), changes in food production (e.g. restricted use of artificial preservatives or additives) and in lifestyle (e.g. more convenience food, less food shopping) have substantially contributed to increasing numbers of food poisoning.

For this reason, there has been a considerable increase in legislation during the past decades covering temperature control requirements and maximum temperatures during the production, distribution and retailing of chilled foodstuffs. However, as soon as a product is taken from a retail display cabinet by the consumer, it is out of any legislation.

Consumer behaviour in handling chilled food has a considerable impact on the shelf life and the quality of the food. A variety of processes along the whole food chain from the retail store to the retrieval of the food from refrigerator at home are involved. In the following sections we review data on: factors affecting consumer behaviour; handling chilled food from shopping to eating; and consumers' perceptions of food quality and safety.

The majority of data presented in the following sections have been obtained from two different online studies ($n=1013$ and $n=1011$) and two subsequent in-home studies of 34 and 82 households in four European countries (Thomas, 2007; Geppert and Stamminger, 2010; Geppert, 2011).

20.6.1 Factors affecting consumer behaviour in handling chilled foods

Food handling in private homes is largely determined by habits and knowledge of the consumers (Flynn *et al.*, 1992). Many studies suggest that a lack of knowledge concerning food safety and foodborne diseases prevents consumers adopting safe food handling behaviour (Leonhaeuser, 1997; Angelillo *et al.*, 2001; Pfau and Piekarski, 2003; Kennedy *et al.*, 2005c). In most cases, children learn about food safety and food handling by observing their mothers. In times of increasing numbers of parents working outside of the home, children often prepare their own food without learning important aspects of proper food handling (Barclay *et al.*, 2003).

According to Fein *et al.* (1995), there is a strong correlation between personal experiences of foodborne illnesses and safe food handling practices; 75% of people changed their behaviour towards a more cautious handling of perishable products after suffering a foodborne disease (Thomas, 2007). People who have never suffered from such diseases are seldom aware of the risks associated with improper food handling. Consequently, they are usually not willing to change their behaviour (Barclay *et al.*, 2003; Thomas, 2007).

20.6.2 Food shopping habits

According to Worsfold and Griffith (1997), the food baskets of European consumers contain up to 60% of chilled and fresh food. Shopping habits as well as transport from retail store to the domestic refrigerator have a considerable impact on the safety and quality of this food (Thomas, 2007). An online study of 1011 households and a subsequent in-home study of 82 households in four European countries yielded data on food shopping pattern (Geppert and Stamminger, 2010; Geppert, 2011). Accordingly, chilled foodstuff is bought at least once per week in more than 90% of survey households, whereas most of them shopped for food once a week (28.0%) or twice a week (27.8%), respectively. Most people in an American (80%) and a Slovenian (74.1%) study stated that they always or often checked packages for damage (Bruhn and Schutz, 1999; Jevsnik *et al.*, 2008). The majority of Slovenian participants (83.9%) usually examined the 'use by' date before purchase, but never checked the temperature of the retail display cabinet (67.8%). The state of frozen food is also important to consumers: 68% of American participants ensured that the food they bought was frozen solid (Bruhn and Schutz, 1999).

After removal from chilled display, unprotected chilled food warms up while shopping and transportation. The increase in temperature depends on both time and ambient temperature.

On average, consumers spend 42 minutes for a bulk shopping trip in grocery stores (Colwill, 1990). In most cases, chilled products are removed from the refrigerated counter within 15 minutes of arrive at the store.

Time taken to transport chilled food from the retail store to the domestic refrigerator was investigated by several authors. Most reported that the majority of consumers took less than 30 minutes to get their food home (James and Evans, 1990; Spriegel, 1991; Worsfold and Griffith, 1997; Jay *et al.*, 1999; Kennedy *et al.*, 2005a; Gilbert *et al.*, 2007; Jevsnik *et al.*, 2008). However, some studies reported that consumers took between 90 and 120 minutes (Evans, 1992; Jay *et al.*, 1999; Kennedy *et al.*, 2005b; Thomas, 2007). According to Thomas (2007), 18% of surveyed households regularly used an isolated bag or cooling box to transport perishable food. In most cases however, foodstuffs were placed in the boot of the car without any protection against heat.

The increase of product temperatures during one-hour transportation was investigated by Evans (1998). A total of 19 different chilled products were monitored in this study. One sample of each product was stored in a pre-cooled insulated box filled with ice packs and the other sample was placed in the boot of the car. The external ambient temperature was within the range 23–27 °C during the tests. Whereas the temperature of the foodstuff stored in the insulated bag remained constant or even slightly decreased, the temperature of the products placed in the boot partially rose up to almost 40 °C. Thinly sliced products were especially affected by a high rise in temperature. After storing in the domestic refrigerator, the foodstuffs required several hours to reach the compartment temperature. That implies a negative impact on both food safety and quality. Predictions by James and Evans (1990) suggest that an increase in bacterial numbers of up to two generations may occur during transport in the boot and subsequent cooling phase in the domestic refrigerator. If foodstuffs are protected against heat by an isolated bag or a cooling box, a bacterial growth of less than 0.4 generations is predicted.

20.6.3   Food handling at home

After shopping, the vast majority of consumers (76%) strived to put perishable foodstuffs into the domestic refrigerator instantly after arrival (Thomas, 2007). Almost 60% of respondents of the same study stated they placed recently purchased items at the back of every shelf behind older foodstuffs (first-in first-out method). Even though recommended storage temperatures are printed on the packages of most chilled products, only 26% of respondents regularly checked these recommendations and complied with them. The decision on where to place certain food products within a refrigerator is frequently based on practical rather than hygienic reasons. Foodstuffs are often placed where space is available or by habit.

Direct observations of European consumers revealed that they seldom made an effort to store perishable items such as raw meat, poultry and fish in the coldest location, which is normally on the bottom shelf of the refrigerator (Geppert *et al.*, 2010). In two-thirds of all cases ($n = 2290$), these products were placed on the top or middle shelves (Figure 20.7). The same study also provided data on packaging of food items. In general, it is recommended to store raw products, especially meat, poultry and fish, in closed containers or to wrap or cover them completely in order to prevent cross-contamination and loss of moisture and flavour. The vast majority of respondents complied with this recommendation. In most cases (87.3%), the original packing was not removed before storage (Figure 20.8). When asked about their practices regarding handling of frozen food, participants of a European online study (Geppert and Stamminger, 2010) responded in quite a different way. About half of the respondents stated that they always used their refrigerator to thaw frozen food (22.7%) or did so if time permitted (24.4%). The other half rarely (28.7%) or never (24.2%) acted this way, which could pose a risk of food safety.
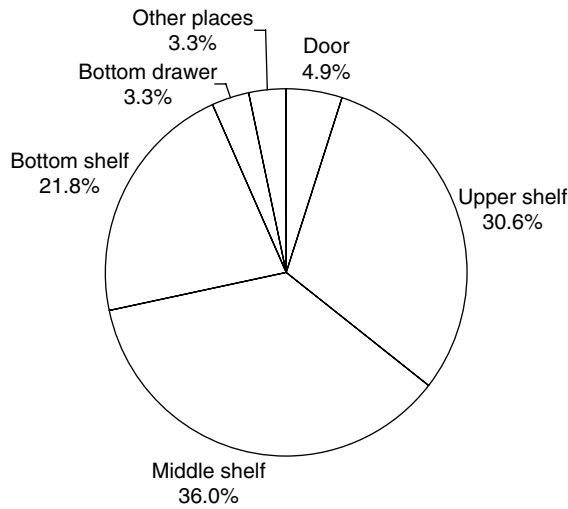
Figure 20.7 Storage places of perishable foodstuffs
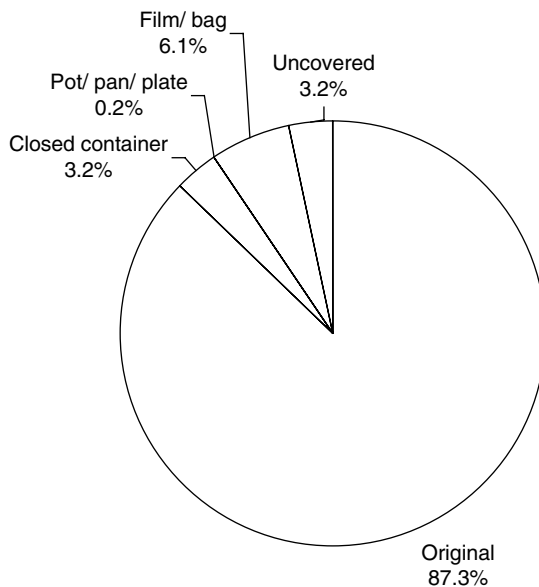
Figure 20.8 Packaging of perishable foodstuffs

20.6.4 Temperatures in domestic refrigeration

One of the most critical factors for the safety of perishable products is the temperature at which a refrigerator is operated in private homes. For this reason, the maximum temperature inside the refrigerator should not exceed 5 °C (Richmond, 1991). Participants of the aforementioned online survey were asked about the temperature they usually operate their main domestic refrigerator. Two-thirds (67.5%) indicated they had a temperature adjustment of 5 °C or below. The remaining third (32.5%) exceeded the recommended temperature range (Figure 20.9). When participants were asked about the reasons for their temperature setting, the most frequent answer was 'it is the right temperature for the food' (Figure 20.10). Several respondents also stated that they maintained their habits or never changed the setting default by the manufacturer. Existing temperature settings were never (40.5%) or sometimes (58.8%) changed. Reasons given for the change of temperature adjustment include the ambient temperature and the amount of foodstuffs loaded in the refrigerator.

More detailed data concerning temperatures in domestic refrigerators were obtained in an in-home study (Geppert, 2011). During this study, temperatures on the middle shelves were recorded by means of a data logger for a period of 11 subsequent days in 82 European households. The overall mean air temperature was 5.5 °C, with 54.4% of refrigerators operating at temperatures higher than 5 °C (Figure 20.11). The highest maximum temperature measured in this study was 16.0 °C, while the lowest minimum temperature in the fresh food compartment went down to −11.5 °C.

Besides natural temperature fluctuations induced by refrigeration cycle, temperature shifts are also caused by frequent or long-term door openings. The effect of door openings on temperature shifts and the recovery time was investigated by Evans *et al.* (1991). Before the opening, the temperature inside the refrigerator was
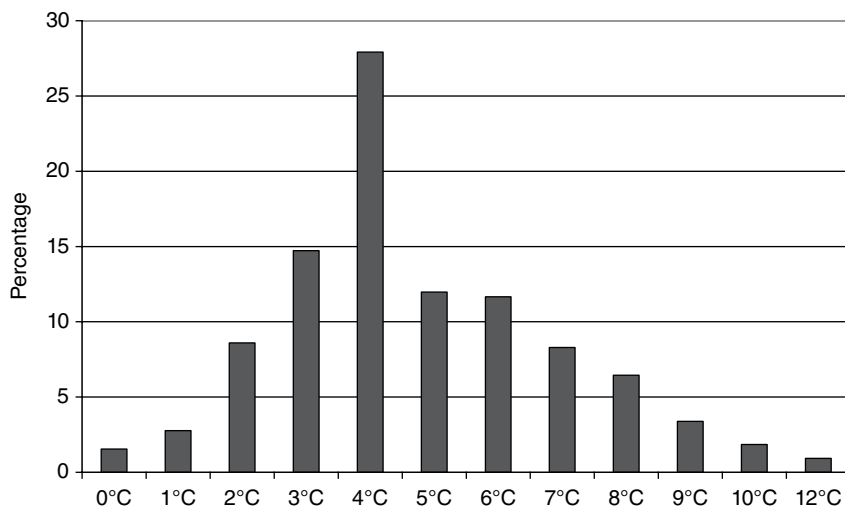
Figure 20.9   Temperature adjustment of domestic refrigerators (source: Geppert, 2011)
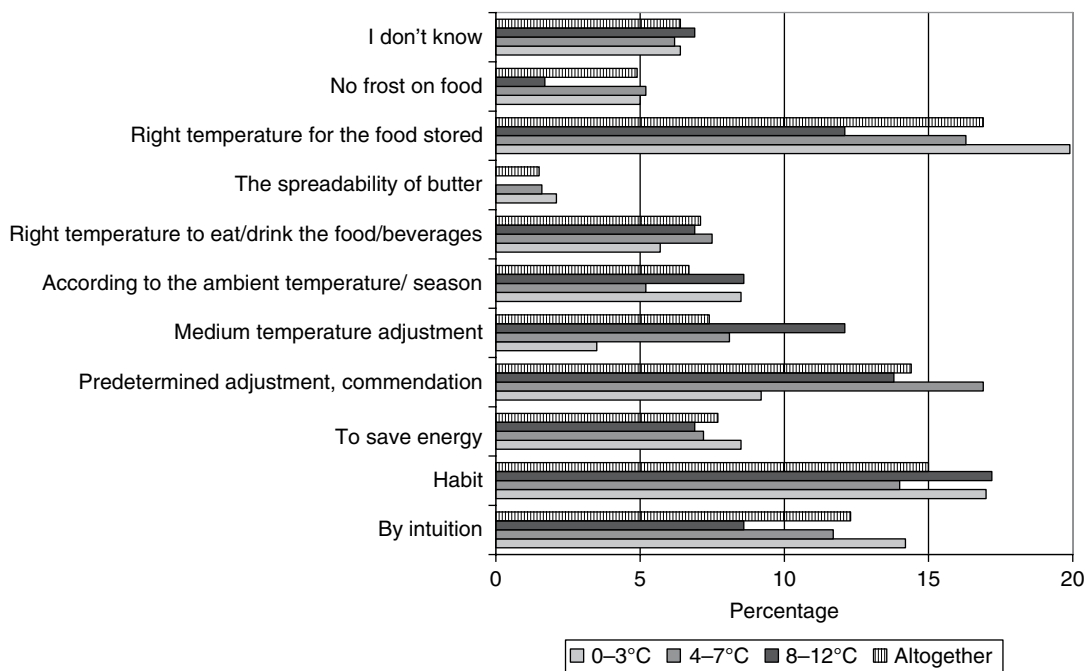
Figure 20.10   Reasons for temperature setting by temperature setting (Geppert and Stamminger, 2010)
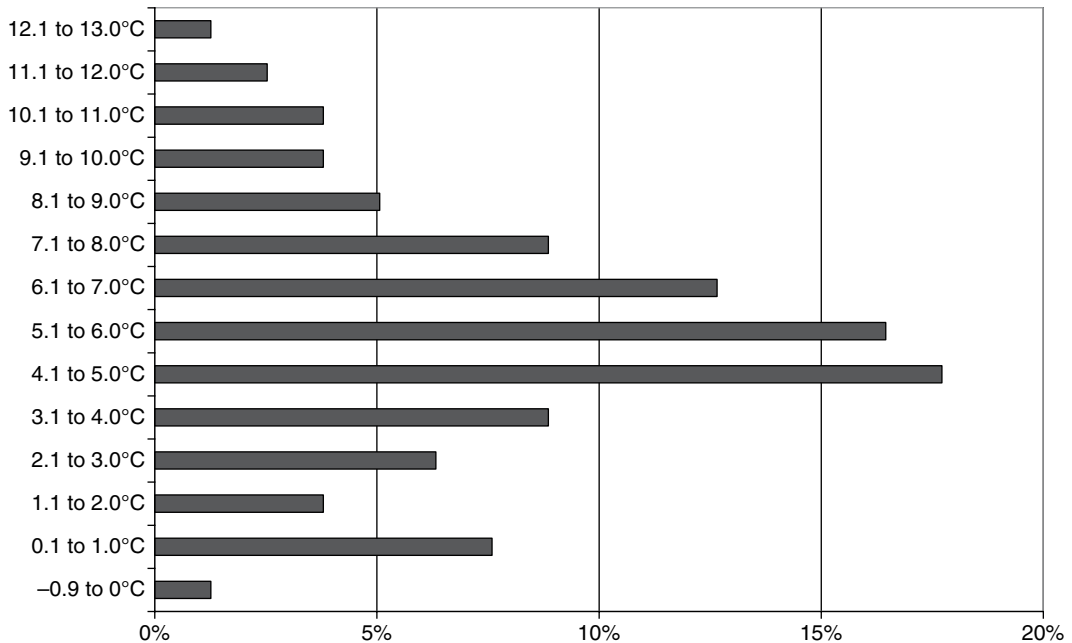
Figure 20.11   Mean air temperatures in refrigerators (measured; Geppert, 2011)

within the range 0–2 °C. After a 3 minute door opening, temperatures rose up to 16 °C depending on the shelf. After door opening, highest temperatures were measured inside the door and at the top shelf, while the lowest values were reached at the bottom of the refrigerator. The air temperatures slowly recovered within a period of about one hour. When subsequent openings occurred, temperatures increased progressively (James and James, 2002). When asked about the daily frequency at which the refrigerator door is opened in their households, most European respondents (78.3%) stated a number of up to 15 times (Figure 20.12).

According to Thomas (2007), the average time the door remained open in European households was 15 seconds. However, durations of up to one minute are quite common. The total time per day totalled 1.5–19.3 minutes, which could have a decisive influence on temperature fluctuations and the quality and safety of the food stored inside.

20.7   Conclusions and future outlook

Due to changes in lifestyle and demography, fresh and minimally processed chilled foodstuffs are on the rise. The present chapter has shown that the quality and safety of these foodstuffs is dependent on innumerable factors. Storage temperature has, without doubt, the highest impact on quality and safety aspects. Even small fluctuations could adversely affect foodstuffs. For this reason, an accurate temperature control and minimized fluctuations in temperatures are absolutely necessary at all stages of the cold chain (from production to consumption). However, the results of various consumer studies have shown that the consumer is often the weakest link in the cold chain. Temperature abuses mainly occur during the transport from grocery store to the home. Additionally, domestic refrigerators are frequently operated at high temperatures. According to predictions, the number of microorganisms can
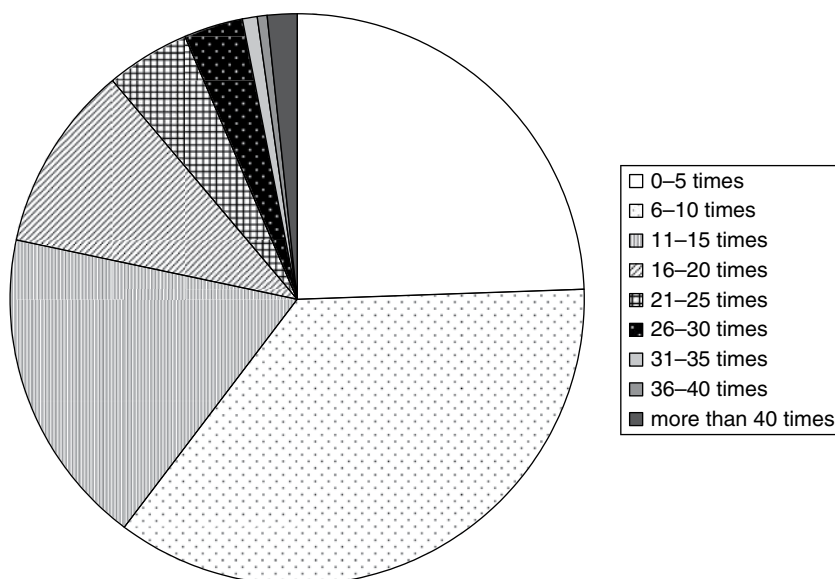
Figure 20.12   Frequency of door openings per day and household (Geppert and Stamminger, 2010)

grow substantially as a result of temperature abuses. In order to improve the microbial and qualitative status of chilled food, manufactures of cooling appliances developed some new features such as temperature and humidity controlled drawers. However, these features are only available in the upper price segment.

The energy efficiency of refrigerators and freezers has been improved drastically during the last decades. As a result, cooling performance has decreased. In particular, highly efficient fixed-speed appliances without special chill compartments have long cycling times and are not particularly sensitive to a rise in internal temperatures. Such appliances might be unable to cope with the complete heat load caused by placing warm foodstuffs inside within a reasonable period of time, even though they are operating continuously. As a consequence, the cooling process of warm food may take a long time and chilled foodstuffs may warm up in the meantime. To date, neither the microbial consequences nor the frequencies and conditions of occurrence of this problem have been analysed; more research is needed on these aspects.

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21

Foodborne Infections and Intoxications Associated with International Travel

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Summary

Issues regarding food safety are of high importance for international travelers. This is especially true while visiting developing countries, where travelers are at risk of acquiring foodborne infections and intoxications. More than 250 different bacterial, viral and protozoan infectious agents are related to foodborne infections. Intoxications occur mostly by the accumulation of toxins in the food chain or by bacterial contamination of foods, with ensuing toxin production. Diarrhea is the most common clinical manifestation of foodborne infections and intoxications, and up to 60% of travelers to high-risk areas experience at least one episode of travelers' diarrhea (TD). Although most episodes are uncomplicated and self-limited, TD can cause significant changes in travel plans such as interruption or discontinuation of holiday trips and business travel schedules. In some patients considerable morbidity and complications may ensue. Severe systemic diseases, such as malaria, can mimic TD and can be lethal if not detected in time.

21.1   Introduction

In a time of globalization, more and more people are traveling internationally for business ventures, visiting their friends and relatives, for recreational activities or other reasons. The number of international tourist arrivals passed the total of 1 billion per year for the first time in 2012 (UNWTO, 2013). As global business is expanding and leisure travelers also want to visit

Table 21.1 Travel associated foodborne infections and intoxications (selection). Asterisk (*) highlights those which may be present without gastrointestinal manifestations.

Viral infections	Parasitic infections
Norovirus	Giardiasis
Rotavirus	Cryptosporidiosis
Hepatitis A, E*	Cyclosporiasis
Poliomyelitis*	Amebiasis
	Toxoplasmosis*
Bacterial infections	*Dientamoeba fragilis*
	Isospora belli
Infection by diarrheagenic E. coli	
Enteric Salmonellosis	
Yersiniosis*	**Helminth infections**
Campylobacteriosis	*Intestinal dwelling:*
Shigellosis	
Aeromonas spp. infection	Anisakiasis
Plesiomonas spp. infection	Ascariasis*
Arcobacter spp. infection	Diphyllobothriasis *
Brucellosis*	Hymenolepiasis
Helicobacter pylori	Taeniasis*
Leptospirosis*	Trichuriasis
Listeriosis*	
Mycobacterium bovis infection*	
Typhoid/paratyphoid fever*	
Cholera	*Tissue dwelling:*
Vibrio vulnificus infection*	
Vibrio parahaemolyticus infection	Angiostrongyliasis*
Intoxications	Clonorchiasis*
	Dracunculiasis*
	Echinococcosis*
Staphylococcal entertoxin intoxication	Fascioliasis*
Bacillus cereus food intoxication	Gnathostomiasis*
Clostridium perfringens food intoxication	Opisthorchiasis*
Botulism*	Paragonimiasis*
Ciguatera*	Toxocariasis*
Tetrodotoxin poisoning*	Trichinellosis*
Shellfish poisoning*	Cysticercosis*
Scombroid*	

remote areas or countries recently opened for tourism, an increasing number of people from industrialized countries are traveling to developing countries, many of which are located in tropical or subtropical regions (de la Cabada Bauche and DuPont, 2011). Here, travelers are exposed to special health risks due to low hygienic standards, weak health systems, and infectious agents which are less prevalent or non-existing in their home countries. A major risk for travel-associated illness is the acquisition of foodborne infections or intoxications. More than 250 etiologic agents have been associated with these disease entities. Not all foodborne infections and intoxications will present as gastrointestinal illness (Table 21.1).

However, diarrhea and other gastrointestinal symptoms are the leading manifestations of foodborne infections or intoxications, and constitute by far the most common health problems experienced by international travelers (Hill and Beeching, 2010, Freedman *et al*., 2006). The resulting clinical syndrome in most cases is defined as travelers' diarrhea (TD). In common speech there exist a variety of colorful names for this disease entity, such as Montezuma`s revenge, Neptune's revenge, or Delhi Belly. Up to 60% of all travelers to developing countries experience at least one episode of TD (Steffen, 2005). Although in the vast majority of cases TD is a benign and self-limiting condition, it can disrupt travel plans and lead to economic loss if occurring during a business trip. Methods to effectively treat TD or to prevent it in the first place are therefore of great importance in the field of travel medicine.

21.2 Travelers' diarrhea

Classic travelers' diarrhea is defined as the passing of three or more unformed stools over a time period of 24 hours with at least one additional symptom such as abdominal pain, abdominal cramps, vomiting, fecal urgency, nausea, or even

fever or blood admixed to the stool. Moderate TD is defined as the passage of one to two unformed stools in 24 hours with at least one additional symptom or the passage of three or more unformed stools in 24 hours without additional symptoms. Mild TD consists of the passage of one to two unformed bowel movements in 24 hours without any additional symptoms (Steffen *et al.*, 1999). In most cases the symptoms of TD will be mild to moderate in severity, but will nonetheless disturb to some extent the planned course of the trip. About 25% of travelers who experience TD are forced to change their travel plans (Hill and Beeching, 2010). The illness will usually be acquired by the consumption of food or beverages contaminated with pathogens, mostly enteritis-causing bacteria. Foods with a high risk of acquiring TD include for example raw or undercooked meat or seafood, raw vegetables, salads, fruits that cannot be peeled, dairy products, ice cream, chipped ice, and tap water (Table 21.2) (Casburn-Jones and Farthing, 2004). The onset of the TD is usually within the first week after arrival and the illness will subside without therapy in most cases, usually after 3–5 days. The risk of acquiring TD is dependent for example on the choice of the travel destination, the season, the type of travel, and the personal tendency for risk-taking behavior concerning food consumption (Casburn-Jones and Farthing, 2004). Additional risk factors include for example the use of antacids like H2-receptor antagonists or proton pump inhibitors, especially in elderly travelers (Neal *et al.*, 1996).

High-risk regions for TD with incidences of 15–50% include the tropical or semitropical areas of South Asia, Africa, the Caribbean (Haiti and Dominican Republic), and Latin America. Intermediate-risk regions are for example China, Russia, and other Caribbean islands with TD rates within the range of 8–15%. In low-risk areas such as countries in Western Europe or Northern America, travelers from other low-risk regions have a likelihood of approximately 4% of acquiring TD (Greenwood *et al.*, 2008).

There exists a range of both host factors and environmental factors contributing to the occurrence of TD. Usually, there is no difference concerning the gender as a risk factor for TD. The age of the traveler plays an important role, as small children and adolescents are especially prone to developing TD. Infants and toddlers tend to put possibly contaminated items into their mouth for exploration, and are therefore at a higher risk of infection (de la Cabada Bauche and DuPont, 2011). In developing countries

Table 21.2 High and low risk foods for the acquisition of TD (Casburn-Jones and Farthing, 2004; DuPont, 2006; Hill and Beeching, 2010)

Foods associated with higher risk of TD	Foods associated with lower risk of TD
Uncooked or raw food (e.g. salad, vegetable, fish, seafood, meat)	Thoroughly cooked, boiled or fried foods that are served hot
Dairy products	Dry foods (e.g. bread, biscuit)
Fruit or vegetables that cannot be peeled (e.g. peach, berries)	Fruits or vegetables that are peeled by yourself (e.g. bananas, apple)
Tap water, unsealed bottled water	Foods containing large amounts of sugar (e.g. marmalade, honey)
Ice cubes, ice creams	Sealed and carbonated bottled water
Sauces standing at room temperature for self-service	Sealed and carbonated bottled beverages
Buying foods from a street vendor	Preparing and washing your food by yourself

diarrheal illnesses are responsible for a high number of deaths in children under 5 years of age (Black *et al.*, 2003). Traveling children may also be endangered by fluid loss or electrolyte disturbances caused by TD. Elderly and more experienced travelers are less prone to suffering from TD, which may reflect some degree of immunity (Steffen *et al.*, 1999). On the other hand, adolescents and young adults are more adventurous concerning their nutrition and are more likely to travel under basic conditions, putting them at increased risk of TD (Alon *et al.*, 2010).

Food bought from street vendors may carry a higher percentage of bacterial contamination. Interestingly, lodging at high-class hotels seems to pose a higher risk of TD than staying in middle-class accommodations (Steffen, 2005). More exclusive hotels and restaurants may prepare exceptional meals containing for example uncooked seafood such as oysters or raw fish, putting the traveler under a higher risk for TD. Generally, the hygiene conditions under which foods are prepared play an essential role in the acquisition of TD. Efforts to improve the hygienic conditions of food preparation and raising the awareness of risk factors of TD have led to marked reductions in the rate of TD in Jamaica, for example (Ashley *et al.*, 2004).

The extent and severity of TD is greatly influenced by the type of causative organism or contaminant. Invasive bacteria or protozoan organisms may cause debilitating and even life-threatening symptoms with fever or bloody diarrhea as hallmarks of invasive disease. Effective chemotherapeutic treatment is often necessary in these cases. Non-invasive organism or toxins can also lead to severe symptoms as profuse watery diarrhea can endanger high-risk groups such as small children, the elderly, or travelers with underlying illnesses. Additionally, life-threatening diseases such as malaria can impose as a case of TD and misdiagnosis can be lethal for the patient (Table 21.3). Therefore, even seemingly 'harmless' episodes of TD have to be evaluated carefully.

21.3   Etiology of foodborne infections

In many cases of TD the responsible agent will be bacteria, viruses or less common protozoan agents. One of the first clues to the prominent role of bacteria in the etiology of TD was that the use of antibiotics by travelers to Mexico effectively prevented the occurrence of TD (Kean, 1963). In most cases, the clinical symptoms will not give a decisive lead to the etiology of TD. Generally, in about 20–40% of cases the etiology of TD cannot be discovered at all (DuPont *et al.*, 1982, 2007). Examination of

Table 21.3   Systemic diseases with diarrhea as symptom

Acute infections	Chronic infections
Avian influenza	African trypanosomiasis
Brucellosis	Chagas disease
Dengue fever	(chronic stage)
Ebola HF	Cytomegaly[b]
Ehrlichiosis	Intestinal tuberculosis
Hantavirus infection	Histoplasmosis
Influenza A (children)	HIV infection
Katayama syndrome[a]	Lymphogranuloma
Legionellosis	venereum
Leptospirosis	*Mycobacterium avium-*
Listeriosis	*intracellulare* infection[b]
Malaria	Schistosomiasis
Marburg HF	(intestinal)
Measles	Visceral leishmaniasis
Ornithosis	Whipple's disease
Plague	
Rickettsiosis	
SARS	
Sepsis	
Tick-borne relapsing fever	
Toxic shock syndrome	
Trichinellosis	
Tularemia	
Viral hepatitis (esp. A and E)	

[a]*Acute schistosomiasis*
[b]*Usually only in immune-compromised patients*
HF: Hemorrhagic fever
SARS: Severe acute respiratory syndrome

native stool samples by microscopy to look for signs of bacterial infection, e.g. leukocytes or erythrocytes in the fecal sample or the detection of protozoan organisms, microbiological methods such as bacterial stool cultures, molecular genetic techniques such as polymerase chain reaction (PCR), or antigen tests are often needed to evaluate the etiology of TD. In the last years a group of pathogens have been determined as frequent causative agents for TD.

21.3.1 *Escherichia coli* (*E. coli*)

Escherichia coli are Gram-negative, rod-shaped, flagellated bacteria belonging to the coliform group of microorganisms which comprise a large part of the physiological gut flora. The bulk of these *E. coli* variants are commensals and involved in infection only if there are aggravating factors such as inoculation to sterile sites, anatomical or functional abnormalities, foreign bodies, or immunosuppression. *E. coli* strains causing intestinal or extra-intestinal disease usually belong to certain pathotypes possessing specific virulence traits based on genes or mobile genetic elements (e.g. plasmids, bacteriophages, transposons) encoding for virulence factors such as toxins, adhesins, colonization factors, invasins, or iron acquisition factors.

E. coli is easily isolated by culture and biochemically characterized. Strains can be differentiated by serotyping using the major surface 'O' (somatic) antigen, a part of the lipopolysaccharide layer, the 'H' (flagellar) antigen, and the 'K' (capsular) antigen. Certain serotypes are associated with specific pathotypes (e.g. O157:H7 with enterohemorrhagic *E. coli*) due to preferred acquisition of mobile virulence elements or frequent phylogenetic traits within a serotype. However, serotyping is not able to definitely identify the pathotype. Therefore, genetic or phenotypic identification of the specific virulence factor is the diagnostic method of choice.

In contrast to intestinal pathogenic *E. coli*, which are obligate pathogens, extra-intestinal pathogenic *E. coli* (ExPEC) are facultative pathogens which belong to the normal gut flora of a certain fraction of the healthy population. Human diseases caused by ExPEC include urinary tract infections, neonatal meningitis, sepsis, pneumonia, and surgical site infections, as well as infections in other extra-intestinal locations.

Intestinal pathogenic *E. coli* (IPEC), which can be divided into at least 6 different pathotypes, is a major cause of diarrhea in children in developing countries leading to significant morbidity and mortality (Makobe *et al.*, 2012). Moreover, diarrheagenic *E. coli* are responsible for a large number of cases of TD.

21.3.2 Enterotoxigenic *E. coli* (ETEC)

The *E. coli* pathotype which has been most often associated with TD is enterotoxigenic *E. coli* (ETEC) (de la Cabada Bauche and DuPont, 2011). In developing countries, it is an important cause of infectious diarrhea. It is estimated that ETEC infections result in up to 650 million cases of diarrhea and 380,000 deaths in children younger than five years of age every year (Steffen *et al.*, 2005). It is transmitted by fecally contaminated food and water. In endemic regions exposed individuals eventually develop immunity, but infection is a risk for non-immune travelers, especially during the rainy season. Additionally, it is also the second most important agent in enteritis outbreaks on cruise ships after infection with norovirus (de la Cabada Bauche and DuPont, 2011). Humans are the most important reservoir for ETEC, but it can also be shed by healthy animals such as cattle and pigs, thereby contaminating the soil and the environment.

ETEC possess specialized pili which are used for attachment to epithelial cells and the bacteria can colonize the small intestine as a first step of the infection. The pili are therefore referred to as colonization-factor antigens (CFAs). ETEC can additionally produce two enterotoxins, which lead to the development of TD. The first is the heat-labile toxin (LT) and the second is the heat-stable (ST) toxin. The bacteria can express LT or ST alone or in combination. ETEC producing both LT- and ST-toxins or ST-toxin alone seem to

cause more severe disease (Qadri *et al.*, 2005) The LT-toxin resembles the Cholera-toxin in structure, function, and antigenic properties. A whole cell/recombinant B subunit (WC/rBS) oral cholera vaccine has shown some protection for TD in infections with LT-positive ETEC. The protection against TD by LT-*E.coli* with WC/rBS cholera vaccination is stated as being up to 60% (Peltola *et al.*, 1991).

There exist two important serogroups for LT-toxin, LT-I and LT-II, with no antigenic cross-reaction. LT-I-producing ETECs cause disease in humans and animals, whereas LT-II is mostly found in isolates from animals but has not been associated with disease. LT-toxin causes diarrhea by activating chloride channels in the intestine. It consists of two different types of subunits, the A and B subunit. After binding of the B subunit to a specific receptor (GM1 ganglioside) at intestinal cells, the A subunit is activated and internalized. Intracellularly, the A subunit increases the level of cyclic adenosine monophosphate (cAMP) via activation of the enzyme adenylate cyclase. This results in a hypersecretion of water and electrolytes into the lumen of the intestine, thereby causing diarrhea.

Concerning the ST-toxin, there exist two different classes of STs referred to as STa and STb. The STa-toxin binds to the guanylin receptor of the guanylate cyclase, as it is similar to the mammalian protein guanylin in structure and function (Wiegand *et al.*, 1992; Carpick and Gariepy, 1993). Binding results in an intracellular increase of cyclic adenosine monophosphate (cAMP), which results in the secretion of chloride and/or the inhibition of sodium chloride absorption, causing diarrhea (Qadri *et al.*, 2005). STb-producing ETECs are mostly isolated from pigs, but can also be found in human isolates. It causes a loss of villus epithelial cells and partial villus atrophy. STb induces the secretion of bicarbonate from the intestinal cells (Sears and Kaper, 1996).

In a systematic review of the etiology of travelers' diarrhea, ETEC was found to be the causative agent in generally 30% of cases. It was especially prevalent in travelers to Latin America and Africa

(Shah *et al.*, 2009). It is transmitted by contaminated food and water. The clinical symptoms include mostly watery diarrhea also associated with abdominal cramping, nausea with or without vomiting, loss of appetite, and headache. Dehydration can be mild to severe. The illness usually lasts 3–4 days after an incubation period of 1–3 days (Qadri *et al.*, 2005).

21.3.3   Enteroaggregative *E. coli* (EAEC)

Another type of *E. coli* which is of major importance in the genesis of foodborne infections is the enteroaggregative *E. coli* (EAEC). The naming stems from the characteristic 'stacked brick' adhesion pattern the bacteria show when in culture with HEp-2 cells. It is an important cause of diarrheal illness in developing but also in developed countries. It's pathogenicity has been shown by the development of diarrhea in volunteers who ingested the 042 strain of EAEC (Nataro *et al.*, 1995). However, there seems to exist genetic diversity concerning the pathogenicity of EAEC and not all strains will cause illness. There are several virulence factors for EAEC including for example the heat-stable toxin-1 (EAST-1) or aggregative adherence fimbriae I, II and III (AAF/I, AAF/II, AAF/III) (Savarino *et al.*, 1991; Nataro *et al.*, 1992; Rich *et al.*, 1999; Bernier *et al.*, 2002).

EAEC causes disease by adhering to the intestinal mucosa, followed by the creation of a mucoid biofilm and the induction of toxic effects on the intestinal mucosa (Flores and Okhuysen, 2009). The bacterium has been associated with diarrhea in Vietnamese children under the age of 2 years (Vu Nguyen *et al.*, 2006). Additionally, it has been found to be a major cause of diarrhea in small children in Brazil (Araujo *et al.*, 2007). In developed countries it has been associated with outbreaks and sporadic disease in several studies for example in Scandinavia, Germany, and Japan (Bhatnagar *et al.*, 1993; Huppertz *et al.*, 1997; Itoh *et al.*, 1997). It has also been described as an important pathogen in the development of TD. In a study examining the pathogens responsible

for TD in travelers with diarrhea in Mexico, Jamaica, and India, EAEC was the second most isolated pathogen, only surpassed by ETEC (Adachi *et al.*, 2001). In a German study on returned travelers with diarrheal illness, EAEC was significantly associated with disease (Paschke *et al.*, 2011). Usually, the pathogen will be acquired by contaminated food and water and a high inoculum is necessary to cause an infection. The clinical symptoms consist mainly of a watery, secretory diarrhea which can also be mucoid. Low-grade fever and little or no vomiting may be present (Bhan *et al.*, 1989; Paul *et al.*, 1994). On the other hand, bloody diarrhea has also been reported in up to one-third of patients (Cravioto *et al.*, 1991).

Infection with EAEC has also been associated with the development of irritable bowel syndrome (Sobieszczanska *et al.*, 2007). In an outbreak of hemolytic uremic syndrome (HUS) in Germany in 2011, the causative bacterium was found to be a Shiga toxin producing *E. coli* which showed virulence factors of both EAEC and enterohemorrhagic *E. coli*, once again displaying the possibility of exchange of virulence factors between different *E. coli* strains or pathotypes (Karch *et al.*, 2012).

21.3.4 Enterohemorrhagic *E. coli*

The enterohemorrhagic *E. coli* (EHEC) is the main cause of the development of hemolytic uremic syndrome (HUS) in children. This life-threatening illness occurs preferentially in children and is defined by the combination of acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia. HUS is usually preceded by a bloody diarrheal illness caused by EHEC, mostly by the serotype O157:H7. The virulence factor responsible for the development of HUS and the intestinal illness is the Shiga toxin (Stx).

There exist two major groups of Stx toxin, classified as Stx1 and Stx2, which do not cross-react immunologically. Stx1 is identical to the Shiga toxin derived from *Shigella dysenteriae* I and is highly conserved. On the other hand there are several different variants for Stx2. The Shiga toxins consist of an A and B subunit, whereby the A subunit is proteolytically cleaved into the A1 and A2 peptides. A pentamer of B subunits binds to a specific glycolipid receptor Gb3 on the surface of eukaryotic cells. The A1 subunit, which is enzymatically active, is bound to the B pentamer by A2 and the holotoxin is endocytosed through coated pits. Intracellularly, the A1 peptide inhibits protein synthesis leading to the death of renal and intestinal epithelial cells. HUS is associated with systemic translocation of Shiga toxins with subsequent activation of the alternate complement pathway, thrombotic microangiopathy, and direct toxin-mediated effects on various cells.

EHEC is transmitted by contaminated food and water but can also spread from person to person. *E. coli*-producing Stx are found in the fecal flora of a wide range of animals, for example, cattle, sheep, pigs, goats, dogs, cats, gulls, and chicken (Griffin and Tauxe, 1991; Beutin *et al.*, 1993; Wallace *et al.*, 1997). The species most important for human infection is cattle. There exist high colonization rates in bovine herds from different countries ranging from 10 to 25%, but also as high as 60% (Griffin and Tauxe, 1991; Wells *et al.*, 1991; Hancock *et al.*, 1994; Burnens *et al.*, 1995). Most infections by EHEC are therefore acquired by the consumption of raw or undercooked contaminated foods, especially stemming from cattle, such as hamburger, roast beef, or raw milk. On the other hand, raw vegetables such as lettuce or sprouts can also be the source of an EHEC infection if they are contaminated with cattle feces (Morgan *et al.*, 1988; Swinbanks, 1996). EHEC infection is mostly thought to be a problem in developed countries but several, also large, outbreaks have been reported from developing countries such as South Africa, Cameroon, or Nigeria, putting travelers at risk (Okeke, 2009). Shiga-toxin-producing *E. coli* have also been found in a small percentage (0.2%) of food handlers in luxury hotels in Kenya (Onyango *et al.*, 2009).

The incubation period usually lasts 3–4 days and the initial illness consists of non-bloody diarrhea with abdominal cramps and short episodes of fever in many patients. Vomiting can also

occur. Bloody diarrhea starts within 1–2 days after the beginning of the illness and lasts between 4 and 10 days. Although most patients will not progress to HUS, it will develop in about 10% of patients that are less than 10 years old and in many elderly patients. The classical triad of HUS consists of hemolytic anemia, thrombocytopenia, and renal failure. Patients can develop oliguria to anuria, edema, pallor, seizures, and other neurological complications.

HUS is treated mostly by supportive care. There is the possibility of administering a monoclonal antibody against complement protein C5, but the re-evaluation of this treatment in the 2011 outbreak in Germany could not confirm a benefit (Menne *et al.*, 2012). There exists the concern of possible adverse effect of treatment with antibiotics on the development of HUS. On the other hand, patients may profit from an aggressive antibiotic strategy (Menne *et al.*, 2012) but this has to be confirmed in further studies. Antimotility agents should not be included in the treatment of diarrhea with EHEC. Additional treatment with dialysis, hemofiltration, and transfusion of erythrocytes and platelets may be necessary. Even with appropriate treatment <5% of children with HUS will die and severe sequelae, for example hypertension, permanent impairment of renal function, or an effect on the central nervous system will occur in 12–30% of the patients in this age group (Pickering *et al.*, 1994; Robson 2000).

21.3.5   Enteropathogenic *E. coli*

The enteropathogenic *E. coli* (EPEC) is a subtype which is of special interest as a pathogen causing diarrhea in infants, especially in the developing world. Damage to the intestine is caused by adherence of the bacteria to the epithelial cells and effacement of the microvilli causing the typical attaching-and-effacing (A/E) histopathology. Loss of the absorptive capacity of the microvilli is probably one method by which the bacterium causes diarrhea by malabsorption (Nataro and Kaper, 1998). The mechanism by which EPEC

causes diarrhea is not finally elucidated. It can inject virulence factors directly into the cytosol of the host cells by using a type-III secretion system (TTSS) (Abe and Nagano, 2000; Kenny, 2002). Additionally, EPEC can introduce its own intimin receptor Tir (translocated intimin receptor) into the host cell membrane (Kenny *et al.*, 1997). Diarrhea is probably caused by the combination of several mechanisms including increased intestinal permeability, active ion secretion, intestinal inflammation, and the loss of absorptive surface because of the effacement of the microvilli (Kaper *et al.*, 2004).

EPEC is transmitted fecal-orally by contamination of foods, hands, or by vomiting (Levine and Edelman, 1984). It mostly causes disease in children under the age of 2 years but may also be pathogenic in adults if ingested in great numbers or after neutralization of the gastric acid (Levine *et al.*, 1978). It has been isolated in outbreaks in healthy adults (Costin *et al.*, 1964; Hedberg *et al.*, 1997) and in sporadic cases in patients with underlying pathology such as diabetes or achlorhydria. In affected patients the pathogen causes watery diarrhea and vomiting, also accompanied by low-grade fever. Prolonged diarrhea and malabsorption may be critical in young children, especially in developing countries (Okeke, 2009). It is rarely isolated from travelers with TD (Gascon *et al.*, 1998; Pommier de Santi *et al.*, 2011).

21.3.6   Enteroinvasive *E. coli*

The pathogenesis of the infection by enteroinvasive *E. coli* (EIEC) seems to be remarkably similar to the infection with *Shigella spp*. EIEC causes disease by invading the colonic epithelial cells followed by lysing of the endocytic vacuoles. It then multiplies intracellularly and, after moving through the cytoplasm, infects nearby epithelial cells (Sansonetti, 1992; Goldberg and Sansonetti, 1993). This leads in the case of severe infection to ulceration in the colonic mucosa by extensive inflammatory responses. EIEC causes disease mainly during outbreaks and transmission is mediated by food or water but can also

occur from person to person (Lanyi *et al.*, 1959; Tulloch *et al.*, 1973; Snyder *et al.*, 1984; Harris *et al.*, 1985). Disease is mostly characterized by watery diarrhea, but can (in a small number of patients) progress to bloody diarrhea resembling dysentery syndrome with bloody and mucoid diarrhea, tenesms, and fever (Snyder *et al.*, 1984; Taylor *et al.*, 1988). Altogether, the incidence of EIEC infection in developed countries seems to be low but outbreaks caused by contaminated foods for example have been described (Gordillo *et al.*, 1992). It is also a rare bacterial cause of TD (Gascon *et al.*, 1998).

21.3.7 Diffusely adherent *E. coli*

The diffusely adherent *E. coli* (DAEC) is inconsistently found as a pathogen causing diarrhea. It was detected for example in an outbreak of diarrheal illness in a French hospital (Jallat *et al.*, 1993) and was associated with diarrheal illness in Brazilian children over the age of 12 months (Scaletsky *et al.*, 2002). The pathogenic mechanism has not yet been finally elucidated. A possible mechanism could be the impairment of the activity and reduction of the abundance of the brush-border-associated dipeptidylpeptidase IV and sucrase-isomaltase (Peiffer *et al.*, 2001). A pro-inflammatory effect has also been proposed (Tieng *et al.*, 2002). It is associated with watery diarrhea without the detection of fecal blood or leukocytes (Poitrineau *et al.*, 1995). DAEC have been found by PCR in up to 11% of patients with TD returning from Guatemala, Mexico, and India, in which no pathogen was detected by standard procedures. It could therefore be a possible causative agent in culture-negative TD patients (Meraz *et al.*, 2008).

21.3.8 Infection by *Campylobacter spp.*

Campylobacter spp. is a Gram-negative spiral-shaped bacterium belonging to the family of *Campylobacteriaceae* which consists of 16 species. It is responsible for a significant number of TD

cases all over the world with 90% of human infections caused by *Campylobacter* (C.) *jejuni* and *C. coli*. The main reservoir of infections is poultry products and it is mostly transmitted by contaminated food which has not been handled properly (Harris *et al.*, 1986; Deming *et al.*, 1987; Adak *et al.*, 1995). Other ways of transmission include the consumption of unpasteurized milk, sausages, or red meat, contaminated water, or contact with pets (e.g. birds and cats) (Kapperud *et al.*, 1992b; Schorr *et al.*, 1994). The infectious dose needed to cause illness showed large strain variation between 800 to 10^9 organisms, and person-to-person transmission does occur more readily with certain strains (Black *et al.*, 1988). In industrialized countries it is often a disease of children under the age of one year and a second peak of infection is found in adults of 15–44 years of age, with males more often affected than females (Friedman *et al.*, 2000). In developing countries this pathogen occurs hyperendemically, especially in children below the age of two years; travelers are also at risk of acquiring the infection.

The illness consists mostly of loose to watery stools but bloody diarrhea can also occur. Additional symptoms are abdominal cramps and pain, fever, and headache. The illness is usually self-limiting, but longer lasting and relapsing diarrhea over several weeks has been reported (Kapperud *et al.*, 1992a). Of concern are complications of *Campylobacter* infections such as the development of Guillain-Barré syndrome which can also occur in persons without clinical symptoms of gastrointestinal infection (Kuroki *et al.*, 1993). Additionally, people positive for HLA-B27 are at risk of developing reactive arthritis (Rautelin *et al.*, 1990). Further complications include the occurrence of cholecystitis, pancreatitis, peritonitis, and gastrointestinal hemorrhage. Meningitis, septic arthritis, endocarditis, osteomyelitis, and also neonatal sepsis have occasionally been reported. *Campylobacter* infection is responsible for a significant amount of TD cases, especially in travelers to South and Southeast Asia (de la Cabada Bauche and DuPont, 2011). Frequencies as high as 25% have been reported

(Hill and Beeching, 2010). There exists the problem of a growing number of quinolone-resistant strains, especially from these regions.

21.3.9   Shigellosis

Bacteria belonging to *Shigella spp*. are responsible for the development of classic bacillary dysentery. It is a Gram-negative non-motile rod, belongs to the family of *Enterobacteriaceae* and is genetically almost identical to *E. coli*. There exist four serogroups of Shigella – *Shigella (S.) dysenteriea*, *S. boydii*, *S. flexneri*, and *S. sonnei* – which are different according to their epidemiology (Ingersoll *et al*., 2002). The bacteria invade the epithelium of the colon after oral ingestion, multiply intracellularly, spread intra- and intercellularly, and kill the cells of the host (Sansonetti, 1991), leading to locally invasive colitis. Additionally, *S. dysenteriae* serotype 1 strains produce Shiga toxin (Stx) with the effect of enterotoxicity, cytotoxicity, and possible neurotoxicity (Donohue-Rolfe *et al*., 1991). Stx is responsible for the development of HUS in patients with Shigellosis.

With humans as the only natural host, the infection is transmitted by water or foods, especially those that can be contaminated by manual processing or preparation and are raw or insufficiently heated before serving (Wu *et al*., 2000). Infected food handlers have been detected as a source of Shigellosis (Al-Lahham *et al*., 1990). The infective dose is very low with 10 bacteria of *S. dysenteriae* and 500 bacteria of *S. sonnei* sufficient for infection, making person-to-person transmission possible (Kothary and Babu, 2001). Children, the elderly, or the immunocompromised are especially at risk. After an incubation period of 1–5 days the infection causes watery to blood diarrhea, abdominal pain with tenesms, headache, fever, and malaise. Possible complications of the infection include the occurrence of seizures, especially in children (Galanakis *et al*., 2002), the development of a toxic megacolon, protein-losing enteropathy, intestinal perforation, HUS, and Reiter's syndrome, especially in HLA-B27 positive persons. The relative occurrence in travelers with TD has been reported to

be as high as 5–15% (Casburn-Jones and Farthing, 2004; Hill and Beeching, 2010).

21.3.10   Salmonellosis

Salmonella spp. are Gram-negative, motile, rod-shaped bacteria belonging to the family of *Enterobacteriaceae*. They can be classified as either the typhoidal serovars (*Salmonella (S.) typhi* and *paratyphi*), which are limited to humans, and the large variety of non-typhoidal *Salmonella* serovars (NTS), which occur in a broad range of hosts and are often transmitted by animals or animal products. NTS are ubiquitous in domestic and wild animals and are one of the main causes of foodborne illness in many countries (Baird-Parker, 1990). NTS mostly cause enteritis but there is also the possibility of invasive disease, especially in immunocompromised patients. A large infecting dose is usually necessary, which means that the bacteria mostly have to multiply in the food before an infection can occur. After an incubation period of 5–72 hours, acute diarrhea occurs which is accompanied by symptoms such as abdominal cramps, nausea, vomiting, malaise, headache, and fever. In the course of the disease the stools can become bloody and mucoid. Sometimes bacteriemia leads to invasive NTS-disease resulting in septicemic illness, typhoid-like illness, or the spreading of bacteria to extra-intestinal organs such as meninges, endocardium, bones, joints, or the liver (Cohen *et al*., 1987). Reactive arthritis may follow an infection with NTS, especially in persons positive for HLA-B27.

The typhoidal serovars *S. typhi* and *S. paratyphi* A, B, and C are the causative agents for typhoid and paratyphoid fever, respectively. The infection is endemic in Southeast and Far-East Asia, especially in the Indian subcontinent, but can also be found in the countries of the Middle East, Central and South America, and Africa. Transmission occurs by contaminated food or water and seldom by person-to-person contact. After an incubation period of 3–60 days the illness begins with continuing high fever in combination with body aches, malaise, headache, general weakness, a dry cough, and abdominal pain (Kollaritsch, 2010).

Typical clinical signs such as 'rose spots' on the abdomen and chest or a relative bradycardia are found in only a minority of patients. The untreated illness usually lasts up to four weeks and can be divided into typical stages. Symptoms at the beginning of the illness commonly include constipation, while diarrhea usually starts in the third week with typical green-yellow stools. The mental state can deteriorate and the patients present in a confused, delirious (typhoid) state. If insufficiently treated, the infection can be lethal due to complications such as intestinal hemorrhage, perforation, or myocarditis. Infections with *S. typhi/paratyphi* are an important differential diagnosis in the returned traveler with fever and systemic illness, especially when returning from Asia (Freedman *et al.*, 2006).

21.3.11 Infection by *Aeromonas spp.*

Aeromonas spp., a Gram-negative rod, has also been identified as a foodborne cause for TD. The genus *Aeromonas* (A.) consists of 14 phenospecies and 17 genospecies, some of which can be pathogenic in humans (Pund and Theegarten, 2008). They can be isolated from fresh- or saltwater and also from fresh and processed foods, for example, raw meat, seafood, fish, and vegetables. The clinical illness consists mostly of watery diarrhea combined with fever and abdominal cramps but seldom with nausea or vomiting (Vila *et al.*, 2003). *Aeromonas spp.* has been associated with TD in 0.8–3% of patients with a predominance in travelers from Asia and Africa (Shah *et al.*, 2009). A tendency for persistent diarrhea that requires antibiotic therapy has been reported (Vila *et al.*, 2003).

21.3.12 Infection by *Plesiomonas spp.*

Plesiomonas (P.) *shigelloides* is a Gram-negative rod that has also been detected as a pathogen in the development of TD. It can be isolated, especially from freshwater samples, but can also be found in river estuaries, seawater, and in fish and shellfish (Medema and Schets, 1993; Shigematsu

et al., 2000). The illness is described as mostly self-limiting and seems to be of short duration, usually lasting about 2 days, and persists over several weeks only in 1% of the patients (Shigematsu *et al.*, 2000). The main symptom is watery diarrhea (Holmberg and Farmer, 1984), starting after an incubation period of about 48 hr (Holmberg *et al.*, 1986). On the other hand, cholera-like illness and signs of acute colitis with abdominal cramps in addition to mucoid and bloody diarrhea have also been reported (Huq and Islam, 1983; Kain and Kelly, 1989). *Plesiomonas* was found in a high percentage of 69.3% in returning travelers to Japan. These travelers arrived mainly from destinations in Thailand, Indonesia, and Vietnam (Shigematsu *et al.*, 2000). In a study on international travelers with TD it was found in up to 13% of travelers to Asia and in up to 6% in travelers to Africa and Latin America (Ericsson, 2003). Most cases described in the literature were reported as sporadic, but epidemics resulting from contamination of water and foods have also been reported (Tsukamoto *et al.*, 1978; Holmberg *et al.*, 1986).

21.3.13 Infection by *Vibrio cholerae* and Non-cholera *Vibrios*

Bacteria of the *Vibrio* species can be found worldwide in the aquatic environment and are more often found in warm waters with temperatures above 17–20 °C (Wright *et al.*, 1996; Heidelberg *et al.*, 2002). Vibrios are highly motile, Gram-negative rods, straight or with a curved shape and a single polar flagellum. Several species are pathogenic for humans, especially *Vibrio (V.) cholerae*, the causative agent of cholera, and other non-cholera Vibrios such as *V. parahaemolyticus*, which also can lead to foodborne gastrointestinal illness (Lee *et al.*, 2001) and *V. vulnificus* that can cause severe wound infections or sepsis, particularly in immunocompromised persons (Blake *et al.*, 1979).

V. cholera is responsible for the development of cholera which can be a rare cause for severe disease in travelers (Chongsuvivatwong *et al.*, 2009). According to the O antigen, the bacterium is divided into more than 200 serogroups (Morris,

2003). The serogroups responsible for epidemic cholera are O1 and O139. The serogroup O1 is further classified into the biotypes 'classical' and 'El Tor' (Sack *et al.*, 2004) and two major serotypes 'Ogaba' and 'Inaba' exist (Longini *et al.*, 2002). *V. cholerae* causes diarrhea by the production of cholera toxin (CT) which leads to increased Cl$^-$ secretion and decreased NaCl absorption by activating the adenylate cyclase (Gill, 1975; Cassel and Pfeuffer, 1978; Gill and Meren, 1978). *V. cholerae* has caused six worldwide cholera epidemics since 1817 and the ongoing seventh pandemic started in 1961 with the El Tor biotype. It is transmitted by contaminated water and foods, especially seafood (Blake *et al.*, 1980; Lowry *et al.*, 1989).

For the O1 serogroup the number of bacteria required to cause the illness has been estimated as 10^5–10^8. Persons with achlorhydria are at risk of infection at even lower numbers of 10^3 (Nelson *et al.*, 2011). The incubation period can last from about 12 hr to 5 days (Morris, 2003; Weil *et al.*, 2009). Most patients infected with *V. cholerae* will display no or mild clinical symptoms. In severe cases (cholera gravis) the fluid loss by watery diarrhea can mount to up to 1 L hr^{-1}. This can lead to severe dehydration, circulatory collapse, and death in the absence of sufficient rehydration therapy. *V. cholerae* can be detected by growth on selective media and is differentiated by biochemical tests and specific antibodies used for serogrouping and serotyping (Qadri *et al.*, 1997). Darkfield microscopy and immunoassays for the detection of CT or the O1 and O139 lipopolysaccharides can also be used for the diagnosis of cholera (Benenson *et al.*, 1964; Almeida *et al.*, 1990; Colwell *et al.*, 1992; Yam *et al.*, 1992; Bhuiyan *et al.*, 2003).

V. parahaemolyticus is also a cause of gastrointestinal disease. It is transmitted mostly by the consumption of contaminated seafood, especially oysters (Daniels *et al.*, 2000b). The incubation period lasts 4–90 hr with a median of 17 hr. The illness manifests as acute gastroenteritis with diarrhea and additional symptoms such as abdominal cramps, nausea, vomiting, and fever, and will continue for several hours to up to 12 days. Dysenteric syndrome has also been reported in association with *V. parahaemolyticus* infection (Hughes *et al.*, 1978) and can be the cause of wound infections and septicemia, especially in immunocompromised patients (Daniels *et al.*, 2000b). It can be isolated for diagnosis on selective media and has been found in travelers with TD in Thailand in up to 9% of cases (Chongsuvivatwong *et al.*, 2009).

V. vulnificus has also been identified as a cause of wound infections, septicemia, and gastroenteritis (Blake *et al.*, 1979; Johnston *et al.*, 1985; Klontz *et al.*, 1988; Park *et al.*, 1991; Shapiro *et al.*, 1998a). Immunocompromised persons and patients with chronic liver disease are at increased risk of developing severe disease. The infection is usually acquired by the consumption of contaminated oysters or by contact with estuarine water.

21.3.14 Infection by *Yersinia enterocolitica*

Yersinia (Y.) enterocolitica is a Gram-negative pleomorphic bacillus belonging to the genus *Yersinia*. It can be divided into more than 60 serotypes of which only a fraction are pathogenic in humans. Infection is acquired by the consumption of contaminated food and water (Keet, 1974; Black *et al.*, 1978). *Y. enterocolitica* preferentially binds to M cells of the Peyer's patches, penetrates these cells and is transported across the epithelial barrier (Autenrieth and Firsching, 1996; Bottone, 1997; Schulte *et al.*, 2000). The bacteria become internalized by macrophages, replicate, and are transported to mesenteric lymph nodes, liver, and spleen (Tabrizi and Robins-Browne, 1992; Viboud and Bliska, 2005). Clinically, enteritis, enterocolitis, acute mesenteric lymphadenitis, and terminal ileitis mimicking appendicitis can develop. There exists the possibility of developing septicemia also in immunocompetent persons. HLA-B27 positive persons are at particular risk of developing reactive arthritis. Diagnosis is performed by culture. *Y. enterocolitica* is a rare cause of TD, but frequencies may be underestimated because of suboptimal isolation methods (Al-Abri *et al.*, 2005).

21.3.15 Infection by *Arcobacter spp.*

Arcobacter spp. is a Gram-negative, spiral-shaped bacterium and belongs together with *Campylobacter spp.* to the family of *Campylobacteriaceae* (Lehner *et al.*, 2005). The most prominent species is *Arcobacter (A.) butzleri* which is also associated with the development of TD. Several other species exist such as *A. cryaerophilus* and *A. skirrowii*, some of which have also been found in human patients. The impact of infection with *Arcobacter spp.* in humans has not yet been finally determined. In travelers to Mexico, Guatemala, and India, *A. butzleri* was isolated in 8% of patients with TD. In this study, a high rate of co-infection with ETEC was also reported. A causative role for *A. butzleri* in TD could therefore not be established (Jiang *et al.*, 2010). Additionally, *Arcobacter spp.* has also been found in blood cultures in patients with high fever and esophageal bleeding and in acute gangrenous appendicitis, which could be a sign of invasive disease (Yan *et al.*, 2000; Lau *et al.*, 2002).

Possible reservoirs for human infection include poultry, but also raw pork and beef meat (Villarruel-Lopez *et al.*, 2003; Morita *et al.*, 2004). A further source for infection could be water including surface and groundwater and also sewage (Musmanno *et al.*, 1997; Jacob *et al.*, 1998; Rice *et al.*, 1999; Stampi *et al.*, 1999; Morita *et al.*, 2004). Furthermore, *Arcobacter spp.* has shown the capability to attach to water distribution pipes (Assanta *et al.*, 2002).

21.3.16 Viruses as causative agents in the development of TD

Viral diseases, especially infection with rotavirus and norovirus, are responsible for a significant morbidity concerning the development of TD and have a worldwide distribution. Rotavirus is a major infectious agent concerning the development of severe and dehydrating diarrhea, especially in infants and young children, around the globe (Leung and Pai, 1988; Cunliffe *et al.*, 2002;

Lee *et al.*, 2003). Infection is associated with the death of over 500,000 persons every year (Parashar *et al.*, 2003; Ramig, 2004). Rotavirus is a double-stranded RNA virus belonging to the family of Reoviridae and is mainly spread by fecal-oral transmission, but transmission by respiratory secretions has also been reported. The non-enveloped virus shows a remarkable stability and can stay infectious on inanimate objects for up to 60 days (Abad *et al.*, 1994). In temperate regions it is mostly transmitted during the cold months (Cook *et al.*, 1990), whereas in tropical regions there is no apparent seasonality. Rotavirus has been found as the cause of TD in up to 36% of travelers with TD in Africa (Ericsson, 2003) but is usually found in up to 10% in affected travelers (Jiang *et al.*, 2002; Pommier de Santi *et al.*, 2011).

The virus infects enterocytes which leads to the destruction of the cells and eventually to the loss of absorptive capacity, leading to malabsorption and diarrhea. After an incubation period of 1–3 days patients experience fever, vomiting, and extensive watery diarrhea which can lead to dehydratation (Katyal *et al.*, 2000; Staat *et al.*, 2002). The illness usually lasts for 5–7 days. The virus can be detected by electron microscopy in the stool, but routine diagnostic is usually performed by enzyme-linked immunosorbent assays and latex agglutination assays (Doern *et al.*, 1986; Nyquist, 1999). Another option is the use of PCR.

Norovirus is another important viral agent for the genesis of gastroenteritis and is the leading cause of non-bacterial gastroenteritis outbreaks (Morillo and Timenetsky Mdo, 2011). It is a non-enveloped, single-stranded, positive-sense RNA virus of the family *Caliciviridae*. Of the five genogroups (GI-V) only GI, GII and GIV are responsible for human infections (Green *et al.*, 1995, 2000; Oliver *et al.*, 2003). It has a worldwide distribution and can be transmitted by contaminated food e.g. frozen raspberries, water, by contact from person to person, fomites, and by contaminated objects (Daniels *et al.*, 2000a; Patel *et al.*, 2008; Glass *et al.*, 2009). It is notorious for causing outbreaks in institutional settings e.g. nursing homes but also on

cruise ships. It has been detected as a cause of TD in up to 10–17% of travelers from different countries (Apelt *et al*., 2010; Hill and Beeching, 2010; Koo *et al*., 2010). Studies reporting prevalences as high as 65% have been published (Chapin *et al*., 2005). Norovirus has been labeled as the third most important cause of TD after ETEC and EAEC (de la Cabada Bauche and DuPont, 2011).

After an incubation period of 1–2 days the illness starts with nausea, vomiting, and watery diarrhea. Accompanying symptoms such as abdominal pain and cramps, malaise, anorexia, and even low-grade fever may be present. The illness is normally self-limiting and usually resolves after 12–60 hr (Morillo and Timenetsky Mdo, 2011). On the other hand, in immune-compromised patients life-threatening illness can ensue (Atmar and Estes, 2006; Dolin, 2007). The method of choice for the detection of the virus is the reverse-transcription polymerase chain reaction from stool samples or emesis (Koopmans, 2008).

21.3.17   Protozoan organisms as cause of TD

Protozoa are single-celled organisms and most of them show an ubiquitous distribution around the world. As a causative agent in the development of TD they play a significantly minor role compared to bacteria (de la Cabada Bauche and DuPont, 2011), which is surprising in contrast to their prevalence in many countries. Usually, transmission occurs fecal-orally and the clinical symptoms may range from asymptomatic carrier status to overt disease; the latter may even require hospitalization, as prolonged and excessive diarrhea might lead to severe dehydration and malabsorption even in immune-competent patients. For the development of foodborne diarrhea in travelers, *Giardia lamblia*, *Cryptosporidium spp*., *Cyclospora spp*. and *Entamoeba histolytica* are of special importance.

21.3.18   Giardiasis

Giardia lamblia is a protozoan organism belonging to the family of *Hexamitidae*. It is recognized as one of the most common isolated parasites in humans. It has a life cycle consisting of two stages which include the trophozoite and the cyst stage. *Giardia* shows a resistance against chlorination and is usually transmitted by contaminated foods and water. After fecal–oral infection with *Giardia* cysts, excystation takes place in the duodenum and each cyst releases two trophozoites. Following asexual reproduction and multiplication of the organism, *Giardia* colonizes the small intestine. Eventually, some of the trophozoites encyst again and are shed by the feces. The trophozoites cause damage to the mucosa, which can lead to clinical disease (Dawson, 2005).

After an incubation period of 1–2 weeks diarrhea will develop. The stools are typically fatty and there are common additional symptoms such as bloating and flatulence. Malabsorption and dehydration can cause significant illness and may lead to hospitalization if the infection is not timely recognized and treated. On the other hand, most cases are self-limiting and will usually resolve in about 5 days. There exists also a large number of asymptomatic carriers. Complications include the development of lactose intolerance, which can lead to diarrhea and bloating even in the absence of *Giardia* infection. *Giardia* has been isolated in approximately 5% of travelers with TD, but is an important cause of persistent diarrhea in travelers (Hill and Beeching, 2010).

21.3.19   Cryptosporidiosis

The genus *Cryptosporidium* consists of several species of which *Cryptosporidium parvum* is the most important as a human pathogen. The reservoirs for this infection are humans and animals and the pathogen can be isolated from a broad spectrum of livestock. As it is even more resistant against chlorination than *Giardia*, contaminated water supplies have been the origin of outbreaks

of cryptosporidiosis; the largest reported was in Milwaukee with an estimated 403,000 cases (MacKenzie *et al.*, 1994). *Cryptosporidium spp.* has a low infective dose and a dose of fewer than ten oocysts have been reported to be sufficient for infection (Okhuysen *et al.*, 1999). After ingestion of the infective oocysts, excystation takes place in the small intestine. The released sporozoites attach to the gut epithelium and further stages of asexual and sexual multiplication follow (Meinhardt *et al.*, 1996). In the end, infective oocysts are shed with the feces. The oocysts can survive well in cool and damp conditions, are widely dispersed in the environment, and can be found for example in surface waters.

The incubation period lasts about 2–10 days. The illness results in watery diarrhea which is accompanied by abdominal pain and vomiting. In immune-competent persons, the illness is self-limiting but may last about 1–2 weeks. Hospitalization is seldom necessary. In contrast, in patients with immunodeficiency (especially HIV-infected patients) the illness may be much more severe and life-threatening. Dissemination in other organ systems, for example the lung or bile ducts, may occur (Farthing, 2000). Infection with *Cryptosporidium* has also been detected in travelers in up to 5% (Ericsson, 2003; Hill and Beeching, 2010).

21.3.20   Cyclosporiasis

The only species of *Cyclospora* pathogenic for humans is *Cyclospora cayetanensis*. There are several different species of *Cyclospora* that can be found in animals. Transmission may occur by consumption of foods that have been in contact with fecally contaminated water. Infections with *Cyclospora* have been associated with foodborne outbreaks through contaminated fresh raspberries, for example (Herwaldt, 2000). After ingestion of infective oocysts, symptoms arise 1–2 weeks later. Patients experience watery diarrhea accompanied by abdominal cramps, nausea, vomiting, weight loss, fatigue, and also fever. Illness is self-limiting in immunocompetent

persons. It will generally resolve in about 2 weeks, but relapses can occur. The prevalence in travelers with TD is reported to be from <1% to 5% (Ericsson, 2003; Hill and Beeching, 2010).

21.3.21   Amebiasis

Amebiasis is an infection caused by the protozoan organism *Entamoeba (E.) histolytica*. It is acquired by the ingestion of infective *Entamoeba* cysts found in fecally contaminated raw or undercooked food and water. Although it was thought that up to 10% of the world population is infected with *E. histolytica*, the discovery of the largely non-pathogenic *E. dispar*, *E. hartmanni* and *E. moshkovski*, which are morphologically indistinguishable from *E. histolytica*, has lead to a different picture of the epidemiology. Various studies using PCR methods for differentiation have shown that *E. histolytica* (sensu strictu) accounts only for a small part (<1–10%) of all *Entamoeba* species found in stool samples (Herbinger *et al.*, 2011). After passing through the stomach and the small intestine, the parasite excysts in the large bowel and the trophozoites multiply there. During further passage in the large bowel the parasite again forms cysts which are shed by the feces and can remain infective for weeks and months in a moist environment. The acquisition of this illness is mainly a problem after travel to countries with poor sanitary standards and travel under simple conditions (Stanley, 2003; Fotedar *et al.*, 2007). Although up to 90% of the infections will remain asymptomatic (Gathiram and Jackson, 1987; Blessmann *et al.*, 2003), clinical symptoms can still arise even after an incubation period of months to years.

Typically, the illness consists of bloody diarrhea and severe abdominal pain and tenesms, the amebic dysentery. On the other hand, watery-mucoid diarrhea or even constipation can result (Fotedar *et al.*, 2007). Elderly or very young patients, pregnant women, malnourished persons or patients receiving glucocorticoid therapy are at risk of severe disease (Fotedar *et al.*, 2007).

Complications of the illness include the formation of strictures, the occurrence of intraluminal masses, so-called amoebomas, rectovaginal fistulas, and perianal skin ulceration. Additionally, bowel obstruction or toxic megacolon with perforation and peritonitis can be lethal (Fotedar *et al.*, 2007). Additional problems arise as there exist the possibility of tissue invasion and the formation of abscesses in different organs, mainly in the liver. This can lead to a life-threatening illness with high-grade fever and right-upper-quadrant pain. Rupture of the abscess into the peritoneum and also into the pleural space or the pericardium is possible. Abscesses can also form in different organs such as the brain, the lungs, the spleen or also in the genitourinary tract (Fotedar *et al.*, 2007). Even patients who are asymptomatic carriers of *E. histolytica* can develop complications by abscess formation. It is usually a rare cause of infection in travelers, but a prevalence of up to 5% have been reported in travelers, especially to India (Jiang *et al.*, 2002; Shah *et al.*, 2009).

21.3.22 Other intestinal parasites as a cause for foodborne infection

There also exists an extensive variety of other protozoan agents and of helminthic infections which can cause foodborne infections after travel. These include for example *Dientamoeba fragilis* or *Isospora belli* and, concerning the helminthic infection, Trichuriasis and Ascariasis have to be mentioned (for other foodborne helminthic infections see Table 21.1). These etiologic agents are seldom found in travelers, but have to be considered especially in long-term travelers or travelers under basic conditions with clinical pathology of longer duration.

21.4 Clinical symptoms/ signs and diagnosis of TD

In most of the cases of classical TD the illness will be self-limiting and the traveler will not seek medical advice or will commence self-treatment.

Clinical illness usually consists of watery diarrhea in combination with additional symptoms like abdominal pain, cramps, fecal urgency, and bloating. Nausea, vomiting, or even fever and blood admixed to the stool may be present. It can be categorized into classical, mild, or moderate TD according to the number of stools in 24 hours and the additional symptoms (see Section 21.2). The clinical signs do not usually differentiate between possible etiologies of TD, but the presence of fever and bloody diarrhea are warning signs of invasive disease. A careful evaluation of the travel history and the medical history can give clues on the possible etiology of TD. Patients with immunodeficiency are for example at risk of developing life-threatening diarrhea by infection with *Cryptosporidium spp.*. Travelers who have lived under simple conditions may be especially prone to bacterial and also parasitic infections.

The type of diarrhea can be clinically discerned into acute watery diarrhea, dysentery, and persistent diarrhea (Casburn-Jones and Farthing, 2004). Acute watery diarrhea is predominated by the passage of watery stools with no or mild fever. Classical pathogens are for example *Vibrio cholerae*, ETEC and EAEC. Viral diseases such as norovirus or rotavirus are also often the cause of this kind of TD. The risk for the traveler consists mainly in dehydration leading to circulatory problems, accompanied by disturbances of the serum electrolytes. Short-duration illnesses accompanied by vomiting and nausea may be the result of intoxication by neuro- or enterotoxins from foods contaminated with *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium perfringens*.

Dysentery is characterized by the presence of bloody diarrhea and fever. It is often accompanied by intensive abdominal pain and tenesms. Typical pathogens include *Shigella spp.* and *Entamoeba histolytica*. Other pathogens such as *Campylobacter spp.*, *Salmonella spp.*, EIEC and EHEC can cause similar symptoms and signs. Complications of inflammatory diarrhea can include severe intestinal inflammation with toxic megacolon, intestinal perforation, and peritonitis in addition to dehydration. Fever in combination with diarrhea may also be a sign of severe

systemic disease with other life-threatening pathogens such as malaria (Table 21.3).

If diarrhea lasts 14 days or longer, it is defined as persistent TD (DuPont and Capsuto, 1996) and protozoan organisms may be considered as the cause. Persistent diarrhea occurs in about 3% of travelers with TD (DuPont and Capsuto, 1996). *Giardia lamblia* is often responsible for persistent diarrhea even in immunocompetent travelers. Infections with *Cryptosporidium spp*. and *Cyclospora spp*. can also lead to prolonged diarrhea in patients, especially in the immunosuppressed (e.g. HIV-positive persons). After an episode of TD up to 10% of affected travelers are at risk of developing irritable bowel syndrome as a sequelae, which can also be the reason for persisting abdominal complaints. Temporary or lasting lactose intolerance after TD is often observed.

Diagnostic procedures can also be guided by the travel history and clinical presentation. Standard examinations which are helpful as a first clue to the pathogenesis of TD include the microscopical examination of a stool smear. This 'native stool' examination can detect the presence of leucocytes or erythrocytes in the stool sample, which can be a sign of invasive intestinal disease. Protozoan organisms such as *Giardia lamblia* or *Entamoeba histolytica* can also be detected by this method. In the case of suspected *E. histolytica* infection, the sample should be taken from the bloody-mucoid part of the stool to improve the result. Further standard procedures include stool sampling for bacterial cultivation on selective media which can discern pathogens such as *Shigella spp*., *Salmonella spp*. and *Campylobacter spp*. Parasitic stool examination by microscopy may need to be repeated as the pathogens are sometimes shed intermittently. Eggs and larvae of intestinal worms can also be found by this method. The differentiation between pathogenic *E. histolytica* and other non-pathogenic *Entamoeba* species can be achieved by the visualization of so-called 'magna forms' with ingested erythrocytes in the amoeba or usually by specific PCR-methods. The diagnosis of *Cryptosporidium spp*. and *Cyclospora spp*. requires special staining of the sample, for exam-

ple, Kinyoun staining. Additional laboratory tests include the application of commercially available stool antigen tests for *Giardia lamblia*, *Entamoeba histolytica* (no differentiation between pathogenic/non-pathogenic species) and *Cryptosporidium spp*. These tests can improve the sensitivity of the stool sampling.

21.5 Therapy of TD

As in most cases of TD, the illness will last only several days and is self-limiting; specific therapy is usually only needed in a subset of patients. Children, elderly people, patients with underlying disorders such as diabetes or cardiac diseases, and immune-compromised persons are at special risk of complication and severe disease. In these groups, greater emphasis should be laid on the detection of the responsible pathogens and the timely initiation of an effective therapy.

The basic treatment of TD includes adequate rehydration therapy. For this, commercially available rehydration solution with appropriate amount and composition of electrolytes and carbohydrates should be used. If oral rehydration therapy is not feasible because of severe dehydration with, for example, signs of neurological impairment or of cardio-respiratory failure or if the necessary amounts of rehydration solution cannot be taken orally because of persisting vomiting, intravenous rehydration may be started. Rapid changes in osmolarity of the blood or of the concentration of electrolytes can lead to life-threatening complications such as cerebral edema or cardiac arrhythmias. Intravenous rehydratation should therefore be carried out by experienced medical personal with regular checks of the essential laboratory values.

Supportive therapy of TD consists of the use of antimotility agents such as loperamide which can effectively reduce the number of stools and improve the consistency. This can prevent substantial interference with travel plans and enable the realization of short trips without the need for the continuous availability of toilettes. Antimotility agents should not

be used in children or in the case of diarrhea combined with fever or bloody stools. Bismuth subsalicylate, an adsorbant medication, is also effective in the therapy of TD and has shown antimicrobial activity in several studies. It should not be used in children because of its salicylate component, and has typical side-effects such as black discoloration of the tongue and nausea. Tinnitus has also been reported in patients taking bismuth-subsalicylate.

Antisecretory medications may also improve the clinical symptoms of TD. Substances such as zaldaride maleat and crofelmer have been shown to reduce the fluid loss but recommendations for their use and approval are still pending. Raceacedotril has been approved as an antisecretory medication in children and adults. A general recommendation for its use in TD is also still pending.

In patients with signs of invasive disease or the detection of leucocytes and erythrocytes, treatment with antibiotics may be considered even if the responsible pathogen has not been isolated. Appropriate medications include azithromycin as it is effective against a broad spectrum of even invasive bacterial pathogens including, for example, *Campylobacter spp*. and *Shigella spp*. Alternative medications are fluorchinolones, especially ciprofloxacin. An increasing number of *Campylobacter spp*. isolates from many different regions of the world are resistant to fluoroquinolones, thereby limiting their use as adjuvant treatment or self-treatment by travelers (Vlieghe *et al.*, 2008). The antimicrobial therapies for the treatment of acute TD are listed in Table 21.4. Another antibiotic which has been shown to be effective in the treatment of TD is rifaximin. It is non-resorbable and can be used against many enteric pathogens including *Campylobacter spp*. As it has practically no systemic resorption, it should not be used in the treatment of invasive disease.

Complications after TD may include the development of Guillain–Barré syndrome and reactive arthritis (especially in HLA-B-27 positive patients after *Campylobacter* infection), the

Table 21.4   Antbiotic treatment for acute travelers' diarrhea (adult dosing regimens)

Drug	Prescription
Ciprofloxacin	750 mg orally as a single dose, or 500 mg twice daily for 3 days[a]
Norfloxacin	800 mg orally as a single dose, or 400 mg twice daily for 3 days[a]
Levofloxacin	500 mg orally as a single dose, or for 3 days[a]
Azithromycin	1 g orally as a single dose, or 500 mg daily for 3 days[a]
Rifaximin[b]	200 mg orally three times daily for up to 3 days, or 400 mg twice daily for 1–3 days

[a] Preferred in enteroinvasive disease (e.g. signs of dysentery and/ or high fever)
[b] Not to be used in patients with signs of enteroinvasive disease

development of hemolytic uremic syndrome in EHEC or Shigellosis, and amebic abscesses in different organs especially liver abscess in *E. histolytica* infection. The risk of developing irritable bowel syndrome after TD has been of growing concern as studies show an occurrence rate of up to 5–10% (Okhuysen *et al.*, 2004; Stermer *et al.*, 2006). TD has also been associated with the onset of inflammatory bowel disease (IBD). It is unclear whether TD can trigger IBD or if the predisposition to IBD may increase the probability of acquiring TD.

21.6   Prevention and Prophylaxis of TD

For many centuries the classical 'mantra' for prevention of TD of all travelers journeying to developing countries has been 'cook it, boil it, peel it or forget it'. Although common knowledge of every traveler, this statement has been questioned concerning its effectiveness and feasibility (Kozicki *et al.*, 1985; Shlim, 2005). Studies on travelers to different regions of the world have shown that this kind of prevention is not nearly enough to protect travelers from the occurrence

of TD (Shlim, 2005). On the other hand, there is growing concern about the sequelae of the 'harmless' illness of TD. Different methods of preventing TD are therefore sought. As most cases of TD are caused by bacterial pathogens, there is a long history of trying to minimize the risk of TD by antibiotic prophylaxis. Medication with ciprofloxacin or norfloxacin has proven to be effective (DuPont, 2007). The possibility that use of a common active antibiotic, potentially for longer periods, may lead to the development of resistant organisms and side-effects including antibiotic-associated pseudo-membraneous colitis has restricted the widespread use of antibiotic prophylaxis in TD. The new medication rifaximin with its missing systemic resorption and positive side-effect profile has provided an option for TD prevention. It has been shown to be 72–77% effective (DuPont, 2007) in the prevention of TD. Other possible substances include the use of bismuth subsalicylate as a preventive measure against TD, which has shown to be 65% effective (DuPont et al., 1980, 1987).

An elegant approach to the prevention of TD would be the development of vaccines. This is of course hampered by the diversity of pathogens in the genesis of TD. Rotavirus vaccines are highly effective in reducing rotavirus enteritis in infants and have been introduced into the standard immunization program in many countries. However, these vaccines have not yet been evaluated in travelers. Various vaccines against noroviruses are in the early stages of clinical development (Atmar and Estes, 2012). The only available vaccine against a bacterial enteric pathogen is the oral cholera vaccine which contains rCTB (recombinant subunit B of the cholera toxin) and killed whole-cell Vibrio cholera O1 of various bio- and serotypes. This vaccine showed a protective efficacy against cholera of c. 85% for a limited period of 6 months (Clemens et al., 1986). It has also been shown to be partially cross-protective against infections with LT-ETEC. The protection rate reaches up to 60% (Peltola et al., 1991) but only against this subtype of ETEC, therefore limiting the use as a general preventive

measure. Further vaccines against TD, especially against ETEC, are in development. Unfortunately, the latest promising candidate, a transdermal patch vaccine against LT-ETEC, has in a large phase III trial not met the expected primary endpoint of reducing the incidence of ETEC infections (Behrens et al., 2013).

21.7 Foodborne intoxications

International travelers are not only at risk of acquiring foodborne infections from bacteria, viruses, or parasitic organisms, but may also experience foodborne intoxications resulting from bacterial enterotoxins in contaminated foods or intoxications from substances which accumulate in the foodchain, which is occurring in the case of Ciguatera. This can result in unpleasant but short-lived gastroenteritis, but can also amount to severe neurologic sequelae with lasting disabilities. The traveler should therefore also be prepared to minimize the risks of such events.

21.7.1 Staphylococcal enterotoxin intoxication

One of the most common intoxications by bacterial enterotoxins results from the ingestion of Staphylococcal enterotoxin (SE). There exist more than 20 types of which SEA and SED are responsible for most of the intoxication with SE (Pinchuk et al., 2010). SE proteins are resistant to heat and acid exposure and are not inactivated by gastrointestinal proteases. SE are mainly produced by Staphylococcus (S.) aureus, but other types of Staphylococcus may also be able to form SE. If foods are contaminated with S. aureus and the bacteria are allowed to grow, then enterotoxin contamination can result. After ingestion of the contaminated food intoxication may ensue. The incubation period is typically short, lasting from several minutes to some hours. The clinical symptoms consist mainly of nausea with vomiting and abdominal pain or cramps followed by

diarrhea (Pinchuk *et al.*, 2010). The diarrhea is self-limiting and rarely dangerous. The incidence in travelers may be greatly under-reported as the illness is usually of short duration.

21.7.2 *Bacillus cereus* food intoxication

Bacillus cereus is an endospore-forming bacteria that can be found in the soil or on plants but can also grow in the intestinal tract of insects and mammals. The adhesive endospores can be found in a wide range of foods including, for example, dairy products, dried fruits, or vegetables with the possibility of cross-contaminating other foods such as meat products (Stenfors Arnesen *et al.*, 2008). After ingestion of contaminated foods two clinical syndromes can result: the emetic disease or the diarrheal disease. The emetic disease is signified by a short incubation period of 0.5–6 hr and results in nausea with vomiting and general malaise. The diarrheal illness usually commences after 8–15 hr with watery diarrhea with abdominal pain and perhaps nausea (Stenfors Arnesen *et al.*, 2008). For both entities, few lethal cases have been reported. The responsible toxins are cereulide for the emetic disease and haemolysin BL, non-haemolytic enterotoxin, and cytotoxin K in the diarrheal disease. As the disease is also self-limiting and usually not problematic, the incidence in travelers is not known but can be speculated to be relevant, similar as in the case of SE intoxication, because of the ubiquitous distribution of these toxins.

21.7.3 *Clostridium perfringens* food intoxication

Clostridium perfringens is an ubiquitous bacterium with natural habitats consisting of soil and also the intestine of warm-blooded animals and humans. It has the ability to form spores and can contaminate foods leading to cases of food poisoning (Brynestad and Granum, 2002). Type A food poisoning results from the production of the Clostridium enterotoxin. After 8–12 hr following the ingestion of contaminated food the illness begins with symptoms including nausea, diarrhea, and abdominal pain (Brynestad and Granum, 2002) and lasts for about 24 hr. Different types of foods have been associated with outbreaks of type A food poisoning such as Mexican food, stew, or corned beef (Hatheway, 1990). Type C food poisoning that can result in life-threatening necrotic inflammation of the small intestine is connected to a lack of intestinal proteolytic enzymes and is currently only found in Papua New Guinea in the context of traditional meals (Brynestad and Granum, 2002).

21.7.4 *Clostridium botulinum* intoxication

Clostridium botulinum is the bacteriological agent responsible for the intoxication named botulism. The bacterium can be found in the soil and also in aquatic sediments and produces 7 toxins (toxin types A–G) which differ immunologically. The toxin types mostly responsible for human disease are the types A, B, E, and seldom F. The toxin effect consists of the blocking of acetylcholine transmission across the neuromuscular junction, resulting in neuromuscular blockade and flaccid paralysis (Sugiyama, 1980; Hauschild, 1989). The ingestion of foods contaminated with botulinum toxin causes foodborne botulism. Growth of the bacteria and the formation of the toxins require certain anaerobic conditions that are found for example in home-canned foods that can be an important source of intoxication (Gangarosa *et al.*, 1971; Shapiro *et al.*, 1998b).

Infants are at risk of developing botulism by colonization of the intestines by *C. botulinum* and absorption of the produced toxin. The clinical entity of infant botulism has been linked to the consumption of honey. Rarely, intestinal production of toxins in adults can produce clinical disease. Wound botulism, with toxin production in anaerobic abscesses, has been linked to IV drug abuse (Werner *et al.*, 2000). The intoxication results in symmetrical cranial nerve palsies and is followed by symmetrical descending flaccid

paralysis that can lead to respiratory arrest (Hughes *et al*., 1981; Shapiro *et al*., 1998b). Gastrointestinal symptoms with vomiting and nausea may be present in foodborne botulism, especially with the toxins B and E. The toxin can be detected from samples of serum, cultures from infected wounds, stool, gastric secretions, or from food samples. Therapy consists of supportive intensive care and antitoxin therapy.

21.7.5 Ciguatera

Ciguatera is the most common type of marine poisoning, which includes a spectrum of diseases acquired by the consumption of marine animals containing toxic substances. The risk areas for Ciguatera include many tropical and subtropical areas in the Indo-Pacific and Caribbean regions. Export of fish and fish products can also lead to cases of Ciguatera in non-endemic countries. The causative agents are ciguatoxins which are formed from toxins produced by the marine dinoflagellate *Gambierdiscus toxicus*. The toxins accumulate in the food chain, starting with herbivorous fish feeding on the dinoflagellates. These fish are then eventually hunted down by carnivorous fish. There exist two classes of ciguatoxin (the Pacific and Caribbean ciguatoxins) which vary in their clinical effect and toxicity (Lehane and Lewis, 2000; Lewis, 2001). The toxins are heat stable and intoxication results in the activation of voltage-sensitive-Na^+ channels, eventually leading to the clinical picture of Ciguatera intoxication (Mebs, 2010a).

After ingestion of herbivorous or carnivorous fish containing ciguatoxins, gastrointestinal and neurological symptoms arise after a variable period of time ranging from less than 1 hr to 2 days. Gastrointestinal symptoms include the occurrence of abdominal pain, nausea, vomiting, and diarrhea. The symptoms can be moderate to severe although the illness is seldom fatal and is mostly resolved in 24 hr. This complex of symptoms is more common in intoxications from the Caribbean. On the other hand, neurological symptoms arise more often after intoxications in the Indo-Pacific areas (Bagnis *et al*., 1979; Lawrence *et al*., 1980; Johnson and Jong, 1983; Gillespie *et al*., 1986). Neurological symptoms consist of muscle pain, paraesthesias, cold allodynia, arthralgia, ataxia, headache, vertigo, and dizziness. These symptoms may last for a prolonged period of time ranging from months to even years. Additional symptoms include pruritus or cardiovascular manifestations. A striking feature of Ciguatera intoxication is cold allodynia with the occurrence of burning sensation when cold objects or water are touched. After the consumption of fish from the Indian Ocean, clinical illness with depression, nightmares, disturbed coordination, and hallucinations have also been reported (Lewis, 2001). There exist reports on the possibility of chronic effects of Ciguatera poisoning with symptoms such as arthralgia, myalgia, chronic fatigue, headache, and pruritus which cannot yet be linked conclusively to the intoxication (Gillespie *et al*., 1986; Goonetilleke and Harris, 2002). Re-exposure to the toxin may also lead to a faster onset of symptoms (Gillespie *et al*., 1986). Although there exists the possibility of detecting ciguatoxin in contaminated fish, the intoxication is mostly diagnosed clinically and by the history of risk exposure. There is no antidote available and the patients have to be treated supportively with adequate rehydration therapy, for example. Symptomatic bradycardia or severe hypotension may need to be treated specifically. A treatment with mannitol has not been proven to be effective in a randomized trial (Schnorf *et al*., 2002). Up to now, the prevention of ingestion of contaminated fish is therefore the most important measure against Ciguatera intoxication.

21.7.6 Tetrodotoxin poisoning

Tetrodotoxin poisoning occurs in most cases after ingestion of inadequately prepared fugu, a fillet of the puffer fish, and is mostly prevalent in Southeast Asia, especially in Japan, where this fish is considered a delicacy (Kanchanapongkul, 2001; Isbister *et al*., 2002; How *et al*., 2003). As the

toxin is unevenly distributed in the body of the fish with higher concentrations for example in the liver or the skin, unskilled preparation might lead to a high risk of intoxication and can lead to fatal food poisoning. The toxin blocks Na$^+$ conductance after binding to the receptor site 1 of Na$^+$ channels, which can lead to conduction failure (Kao, 1966).

Depending on the amount of toxin ingested, in mild cases sensory symptoms such as perioral numbness or numbness at the distal limbs accompanied with paraesthesia develop within 60–90 min (Torda *et al.*, 1973; Kan *et al.*, 1987; Tibballs, 1988; Isbister *et al.*, 2002). Gastrointestinal symptoms with nausea and vomiting may also occur.

Moderate illness is characterized by distal muscle weakness and weakness of bulbar and facial muscles. Eventually, ataxia and disturbed reflexes may develop. Severe poisoning results in a generalized flaccid paralysis accompanied by respiratory failure. The patients are conscious but experience aphonia and the pupils are fixed and dilated (Torda *et al.*, 1973; Tibballs, 1988). In life-threatening intoxication bradycardia, dysrhythmias and hypotension may occur and respiratory failure and coma may ensue. The duration of symptoms is also dependent on the degree of intoxication, with moderate to severe cases usually resolving after 5 days. A longer duration is found in severe intoxication (Torda *et al.*, 1973; Tibballs 1988; Isbister *et al.*, 2002). The intoxication is diagnosed clinically, but material from uneaten fish and the urine and serum of the patient can be examined for tetrodotoxin (Mebs, 2010c). There exists no antidote, and therapy consists of supportive treatment including intensive care measures if necessary.

21.7.7 Paralytic shellfish poisoning

The paralytic type is the most common type of shellfish poisoning and has an average mortality rate of 6% (Lehane, 2001; Mebs, 2010b). It is acquired by the ingestion of bivalve shellfish including oysters, clams, and mussels that are contaminated with saxitoxin or with derivates such as gonyautoxins. The toxins stem from marine microalgae dinoflagellates such as *Gymnodium catenatum*, *Alexandrium spp.*, and *Pyrodinium bahamense var. compressum*. These organisms are consumed by the bivalves and the toxin accumulates (Lehane, 2001). Shellfish-eating crabs may also be a source of intoxication (Llewellyn, 2001; Llewellyn *et al.*, 2002). The most important paralytic shellfish toxin is saxitoxin.

The highly potent neurotoxin blocks the tetrodotoxin-sensitive Na$^+$ channels, which leads to disruption of the nerve conduction and neurological symptoms (Kao, 1966; Ritchie and Rogart, 1977). After a latency period of 30 min to 3 hr, paraesthesia occurs, especially at the tongue and lips and also at the face and extremities. The paraesthesia then generalizes and ataxia and weakness of the arms and legs develops. Additional symptoms include nausea with vomiting, headache, hypersalivation and also diaphoresis. There is no loss of consciousness. In severe cases there is a rapid progression to paralysis and respiratory failure (Mebs, 2010c; Lehane, 2001). The toxin can be detected by bioassays or immunoassays (Usleber *et al.*, 1997, 2001). The treatment is supportive; an antidote for saxitoxins is not available for clinical use.

21.7.8 Neurotoxic shellfish poisoning

This kind of shellfish poisoning can be found in certain areas including the west coast of Florida, North Carolina, and New Zealand (Morris *et al.*, 1991; Ishida *et al.*, 1996; Mebs, 2010c). The responsible toxins are brevetoxins produced by the marine dinoflagellate *Gymnodium breve* which is taken up by the shellfish. The toxins lead to nerve-cell depolarization and spontaneous firing by enhancing the Na$^+$ entry (Baden, 1989). Neuroexcitatory symptoms result and patients experience gastrointestinal symptoms such as nausea, abdominal pain, and diarrhea in addition to neurological symptoms such as myalgia,

vertigo, ataxia, and paraesthesias. The symptoms are similar to those in ciguatera but are mostly mild; supportive treatment is sufficient.

21.7.9 Amnesic shellfish poisoning

Amnesic shellfish poisoning is a toxic encephalopathy caused by the ingestion of mussels containing domoic acid, a neuroexcitatory amino acid similar in function to glutamic acid. It is heat stable and produced by microscopic algae of the genus *Nitzschia*. After an incubation period of about 6hr, the clinical disease starts with gastrointestinal symptoms such as abdominal cramps, vomiting, and diarrhea. After 48hr the patients may develop neurologic symptoms consisting of confusion, disorientation, mutism, seizures, myoclonus, coma, disorders of eye movements, and loss of short-term memory (Perl *et al.*, 1990). Additional symptoms include cardiovascular symptoms and strong respiratory secretion. Older patients may be at risk of severe and also lethal disease. In severe intoxication, neurologic symptoms may be ongoing for example with anterograde amnestic syndrome (Teitelbaum *et al.*, 1990). The intoxication can only be treated by supportive means. The toxin can be detected by high-performance liquid chromatography and a mouse bioassay (Perl *et al.*, 1990).

21.7.10 Scombroid

Scombroid intoxication is caused by histamine and other scombrotoxins. These substances are produced by bacterial enzymes in decomposing fish meat which has been inadequately stored or refrigerated. These bacteria include for example *Klebsiella pneumonia*, *Proteus morganii* or *E. coli* (Barbier and Diaz, 2003). The name 'Scombroid' stems from one source of intoxication which are fish belonging to the family of Scombridae, e.g. tuna or mackerel. On the other hand, intoxication is mainly found in association with the ingestion of non-scromboid fish such as sardine, anchovies, herring, mahi-mahi, Australian ocean salmon, kahawai, or bluefish (Bean *et al.*, 1996).

The symptoms of intoxication start minutes after the ingestion and typically consist of facial flushing, headache, tingling of the mouth, burning sensation of the throat, cardiovascular symptoms, and gastrointestinal symptoms such as abdominal pain, nausea, vomiting, and dysphagia. Pruritus, urticaria, and bronchospasms with wheezing may also be present. Signs and symptoms may yield clues to the diagnosis but can be confirmed by elevated histamine levels in urine and serum. The treatment includes antihistamines and corticosteroids or epinephrine may also be necessary. The intoxication is self-limited and no lethal outcomes are known of (Barbier and Diaz, 2003). In severe cases, induced vomiting with syrup of ipecac or gastric lavage with sub-sequential administration of activated charcoal may be indicated.

21.8 Conclusion

Foodborne illnesses and intoxications are common health problems in international travelers and are caused by a large spectrum of different pathogens and toxins. Most cases present with diarrhea and other gastrointestinal symptoms and have a self-limited and uncomplicated course. However, in some patients severe and complicated disease can evolve, especially in the very young, the old, the immunocompromised and in patients suffering from underlying conditions. In addition, febrile diarrhea in travelers might be the initial presentation of other potentially dangerous diseases such as malaria. Some foodborne intoxications lead to neurological disease which – in the absence of gastrointestinal symptoms – can be a diagnostic challenge.

The treatment of diarrhea in travelers is mainly symptomatic using oral rehydration or intravenous rehydration in severe cases. Antibiotic treatment with quinolones or azithromycin is justified in enteroinvasive disease which is mainly caused by bacterial pathogens and often presents with fever and erythrocytes and leukocytes in the stool. With the exception of antitoxin therapy for botulism, the treatment of foodborne intoxications is only supportive.

So far, the prevention of foodborne illnesses and intoxications mainly relies on the avoidance of risky food and water. Currently, only a few vaccines are available such as oral rotavirus vaccines for infants and the oral cholera vaccine which only provide limited protection. New vaccines against norovirus and other enteropathogens are under development.

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22
Electron Beam Inactivation of Foodborne Pathogens with an Emphasis on *Salmonella*

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Summary

Electron beam (e-beam) irradiation is a non-thermal alternative to heat processing. This technique inactivates microorganisms, viruses, and insects that might be present in a food by generating radiation using accelerated electrons. The mechanism of inactivation for microorganisms by e-beam is believed to be due to the direct interaction of the radiation with cell components and also due to the indirect action with free radicals generated by water radiolysis. E-beam irradiated food remains generally unaffected in texture, taste, and nutritional value. It is approved by the Food and Drug Administration for use on meat, poultry, spices, fresh fruits, and vegetables. Recent outbreaks of foodborne salmonellosis in foods not typically heat-treated have given the food industry cause for concern. Since e-beam does not use heat to minimize microbial hazards, the use of e-beam as a non-thermal preservation method for fresh and ready-to-eat foods is of interest. The purpose of this chapter is to review the application of e-beam irradiation to inactivate foodborne pathogens, with an emphasis on *Salmonella*.

22.1   Introduction

Despite advances in food processing technology, food handling, and food safety awareness, foodborne (FB) illnesses remain common in the United States. Most pathogenic agents are transmitted by a variety of vectors and can enter the food supply chain at any number of places. Linking pathogens of a specific illness to a particular food or ingredient is therefore difficult except during an active outbreak. The Centers for Disease Control and Prevention (CDC) most recent estimates indicate that 1 in 6 people (roughly 48 million) in the US will get sick, 128,000 will be hospitalized and 3000 will die of foodborne illnesses (CDC, 2013). These new estimates were generated using public health surveillance systems such as the Foodborne Diseases Active Surveillance Network (FoodNet) and surveys to assess trends for which illnesses are increasing as well as which illnesses are decreasing. Estimates from 2011 show that the top five pathogens contributing to domestically acquired foodborne illnesses resulting in death included *Salmonella* (28%), *Toxoplasma gondii* (24%), *Listeria monocytogenes* (19%), Norovirus (11%), and *Campylobacter spp*. (6%) (CDC, 2013). The top five pathogens are consistent with those previously reported by Mead *et al*. (1999) and the newer statistics show lower frequencies of FB illness. Unfortunately, the long-term trend for illnesses by non-typhoidal *Salmonella* indicates that they are increasing (CDC, 2013).

FoodNet confirms that *Salmonella* continues to be the leading bacterial cause in terms of the number and incidence of laboratory-confirmed FB illnesses in the US (CDC, 2013). *Salmonella* is estimated to cause over 40,000 cases and 400 deaths each year in the US alone (CDC, 2013). Illness from *Salmonella* infection is a global concern; for example, there were 108,614 human cases of salmonellosis reported in the European Union in 2009 (EFSA, 2011).

Salmonella species are Gram-negative facultative anaerobic rod-shaped, non-spore forming, motile bacteria which belong to the family of *Enterobacteriaceae*. They were first discovered in 1885 when Theobald Smith, a research assistant for Daniel E. Salmon, discovered the first strain of *Salmonella* and named it *Salmonella choleraesuis*. Since then, over 2300 *Salmonella* strains (typically referred to as serotypes or serovars) have been confirmed to cause salmonellosis. The types of salmonellosis fall into two main categories: typhoid or non-typhoidal. *S. typhi*, the strain that causes typhoid fever, is transmitted via fecal–oral route from infected to healthy individuals. Infection by *S. typhi* is uncommon in the US as it is largely associated with poor hygiene and contaminated water and food supplies. Many of the pathogenic non-typhoidal *Salmonella* are in the species '*enterica*' which has six subspecies: *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *indica*. Serovars from the subspecies '*enterica*' are the most frequently identified agents of FB illness in the US. Recent *Salmonella enterica enterica* outbreaks implicated various serovars as the causative agents of illness, such as Bredeney, Tennessee, and Typhimurium associated with peanut butter, Braenderup associated with mangoes, Typhimurium, Newport, and Panama associated with cantaloupe, and Heidelberg, Hadar, and Montevideo associated with poultry, and so on (CDC, 2013).

Salmonella spp. are adaptable to various environmental conditions including high acid, antibiotics, and antimicrobial drugs (Foster and Spector, 1995). Because they are so adaptable, some strains have become antimicrobial-resistant; strains that are resistant to multiple antimicrobial drugs (multidrug-resistant or MDR) are an emerging issue to public health (Musgrove *et al*., 2006; Alcaine *et al*., 2007). MDR strains of *Salmonella* are associated with a higher frequency of causing illness, longer hospital stays, longer duration of illness, and a two-fold increase in the risk of death in the two years after infection (EFSA, 2009). *Salmonella* cells with enhanced acid tolerance will more likely survive gastric acidity and enter the small intestine and cause illness; people taking antacids to reduce gastric acidity are at a much greater risk of this type of infection (Banatvala *et al*., 1999; Smith, 2003). Similarly, individuals who have been

treated with antibiotics may also be more easily infected because of the disturbance of natural flora in the human gut, which normally provides some protection against *Salmonella* (Berends *et al.*, 1998; Asahara *et al.*, 2001; Lee *et al.*, 2003).

Refrigeration, heat, and treatments that lower pH and/or water activity (a_w) are typical strategies employed by the food industry to halt pathogenic bacteria such as *Salmonella* from growing in foods. Unfortunately, it is now apparent that although these conditions can inhibit cell division, they do not stop bacterial growth or chromosome replication. Bacterial growth continues with the formation of long (>200 μm) multinucleate filaments that will possibly cause rapid division of the cells when the conditions become more favorable to growth (Mattick *et al.*, 2003). This action was confirmed when *S*. Enteritidis and *S*. Typhimurium were grown in low a_w foods at low temperatures (Mattick *et al.*, 2003). Peanut butter, a low a_w food, has been implicated with multiple outbreaks of salmonellosis, with the most recent outbreak occurring in 2012 (CDC, 2013). These outbreaks highlight the need to explore other means to control this pathogen beyond the typical strategies used by the food industry. The development of new and efficient controls aimed at significantly reducing foodborne salmonellosis should be a multi-faceted and multi-disciplinary effort by universities, governmental agencies, and the food industry. Electron beam (e-beam) irradiation is a viable alternative to thermal processing, especially if the commodity is ready-to-eat (RTE), minimally processed, or consumed uncooked such as peanut butter, fruits, and vegetables.

E-beam radiation does not require radioactive isotopes, unlike gamma radiation, to generate ionizing radiation to inactivate bacteria in food products. Instead, the ionizing radiation is generated by an electrical device called a linear accelerator which is able to increase the kinetic energy of electrons. When these excited electrons penetrate the food product they are able to cause microbial death. Although gamma radiation and e-beam radiation have similar effects on food, it is possible that consumer acceptance of this technology would be more favorable towards e-beam because of the negative association of gamma radiation with the nuclear industry.

22.2 Food irradiation

The safety of minimally processed and fresh foods is a concern that the food industry has responded to by actively seeking and developing new non-thermal technologies (i.e. e-beam, gamma, UV, high pressure, pulsed electric field, high intensity light, and plasma discharge) that could assure the same high food safety standards as heat processing, but without the changes in quality characteristics associated with heat. More importantly, there have been many recent bacterial foodborne illness outbreaks associated with commodities that simply cannot be heat treated at the commercial level because they would lose their 'fresh' symbol of identity; for example, fresh meat, fish, poultry, ready-to-eat (RTE) foods, fruits, and vegetables would be dramatically altered (cooked) if exposed to thermal treatments sufficient to inactivate pathogenic organisms. Irradiation of foods is considered a 'cold pasteurization/sterilization' process because there is a negligible change in product temperature, thereby killing pathogens and extending the shelf-life without cooking the product.

Pharmaceutical products and plastic laboratory and medical equipment have long been sterilized by ionization radiation. When food is irradiated, ionizing radiation or energy is used to treat foods in order to kill organisms to increase product safety and reduce spoilage. It is an effective method for microbial reduction and has been used by some segments of the food industry since 1963 (USDA, 2012). Energy exists in waves and its properties are defined by its wavelength; for example, radio communication, television, and microwaves occur at the long-wave end (low-frequency) of the spectrum whereas ionizing radiation such as e-beam, X-ray, and gamma rays are at the short-wave end (high-frequency) of the spectrum. The range of energy frequencies and their respective wavelengths are identified on the

Food irradiation

Wavelength (meters)	Radio	Microwave	Infrared	Visible	Ultraviolet	X-ray	Gamma ray
	10^3	10^{-2}	10^{-5}	$.5 \times 10^{-5}$	10^{-8}	10^{-10}	10^{-12}

About the size of...

| Buildings | Humans | Honey bee | Pinpoint | Protozoans | Molecules | Atoms | Atomic nuclei |

Frequency (Hz)

10^4 10^8 10^{12} 10^{15} 10^{16} 10^{18} 10^{20}

Energy of one photon (electron volts – eV)

10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5} 10^{-4} 10^{-3} 10^{-2} 10^{-1} 1 10^1 10^2 10^3 10^4 10^5 10^6

Lower energy   Higher

Figure 22.1   Food irradiation and electromagnetic spectrum.

electromagnetic spectrum (Figure 22.1). At higher frequencies (or shorter wavelengths) there is an increase in energy. Ionizing radiation exists at the shortest wavelengths and is able to excite and ionize atoms when they interact with matter. In order to be considered 'ionizing' radiation, enough kinetic or quantum energy is needed to induce a valence electron to leave an atom, thereby ionizing it. The Codex Alimentarius general standard for irradiated foods identifies three principle types of radiation sources: gamma rays, produced from radio isotopes cobalt-60 (1.17 and 1.33 MeV) and cesium-137 (0.662 MeV); x-rays generated from machine sources (maximum energy 5 MeV); and electron generated from machine sources (maximum energy 10 MeV) (FAO, 2003). The general standard also recommends that the absorbed dose should not exceed 10 kGy (FAO, 2003).

As more food items, including grains, meat, and fish products, are approved by United States Department of Agriculture (USDA) and FDA to be treated with radiation for the reduction of spoilage and pathogenic microorganisms, food irradiation is expected to be a major factor in food safety and preservation strategies in the coming years (Turman *et al.*, 2003). Ionizing radiation of foods is gaining acceptance in Europe as well as Asia–Pacific region in order to meet sanitary and phytosanitary requirements in the international market (Luckman, 2002; Kume *et al.*, 2009). The number of countries that recognize irradiation as a useful technology for the reduction of pathogens for public health significance as well as part of overall good manufacturing practice (GMP) and hazard analysis critical control points (HACCP) systems is increasing (Luckman, 2002). The CDC estimated that if the US irradiated 50% of poultry, ground beef, pork, and processed meat, there would be nearly 900,000 fewer cases and 350 fewer deaths each year due to FB illnesses (Tauxe, 2001). It is estimated that the American consumer would receive approximately $2 in benefits such as reduced spoilage and less illness for each $1 spent on food irradiation (Loaharanu, 2003).

22.3   Inactivation of *Salmonella* with e-beam and ionizing radiation

22.3.1   Application of electron beam

Food processing by e-beam radiation pasteurizes and, depending on dose, sterilizes foods by generating high-energy electrons which are accelerated to the speed of light using a linear accelerator. These high-energy electrons are then transferred to the e-beam 'gun' which subsequently passes them onto the product, resulting in microbial inactivation (Figure 22.2). The source of electrons comes from electricity and, unlike gamma radiation, the e-beam does not use radioisotopes. The overall antimicrobial properties of gamma radiation and e-beam are similar; however, the processing times associated with e-beam are shorter due to the ability to apply higher dose rates (e-beam: 10^3–10^5 Gy s^{-1}; gamma: 0.01–1 Gy s^{-1}). A major limitation of e-beam is that it has a limited penetration depth. The high-energy electrons can effectively penetrate approximately 8–10 cm in typical food products (i.e. specific density 1 g cm^{-3}) whereas there are no limitations with gamma radiation (Jaczynski

and Park, 2003a). The size and dimensions, as well as specific density of food products, should therefore be carefully considered prior to e-beam processing.

E-beam 'dose' is a measurement of the amount of radiation energy absorbed by the food as it is subjected to e-beam processing. Film dosimeters are used to measure the actual radiation dose applied to products. Dosimeters are small, typically very thin, radiation-sensitive films that measure actual electron penetration throughout the product. The dose is measured in Grays (G) or kiloGrays (kGy), where 1 Gray = 0.001 and 1 kGy = 1 Joule (J) of energy absorbed per kilogram (kg) of irradiated food. As a food product is subjected to e-beam radiation, the energy (kJ) is deposited (i.e. absorbed) within the product. Some of the absorbed energy is converted to heat. For example, if a food product is processed at 10 kGy, then the product will absorb about 10 kJ or an equivalent of 2.4 kcal of energy. If we also assume that all of the absorbed energy is converted to heat, then there will be a 2.4°C (4.3°F) rise in temperature of the product. The dose of 10 kGy in this example exceeds the highest dose approved for meat products; typically, lower doses are used for food products. The quality changes caused by this temperature rise would
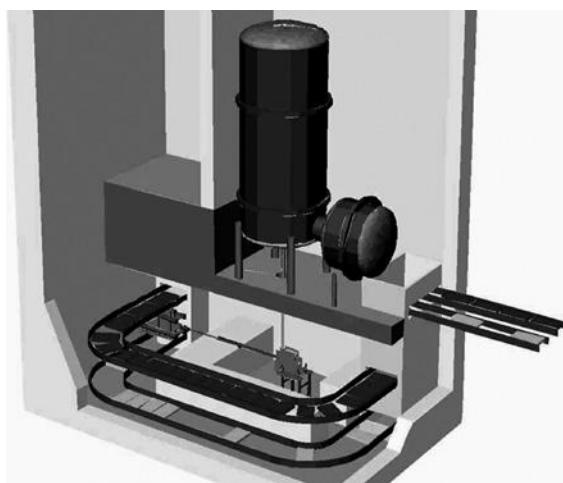
Figure 22.2   In a single-sided e-beam configuration, the linear accelerator delivers fast electrons via the e-beam gun from one side.

(a)

(b)

(c)
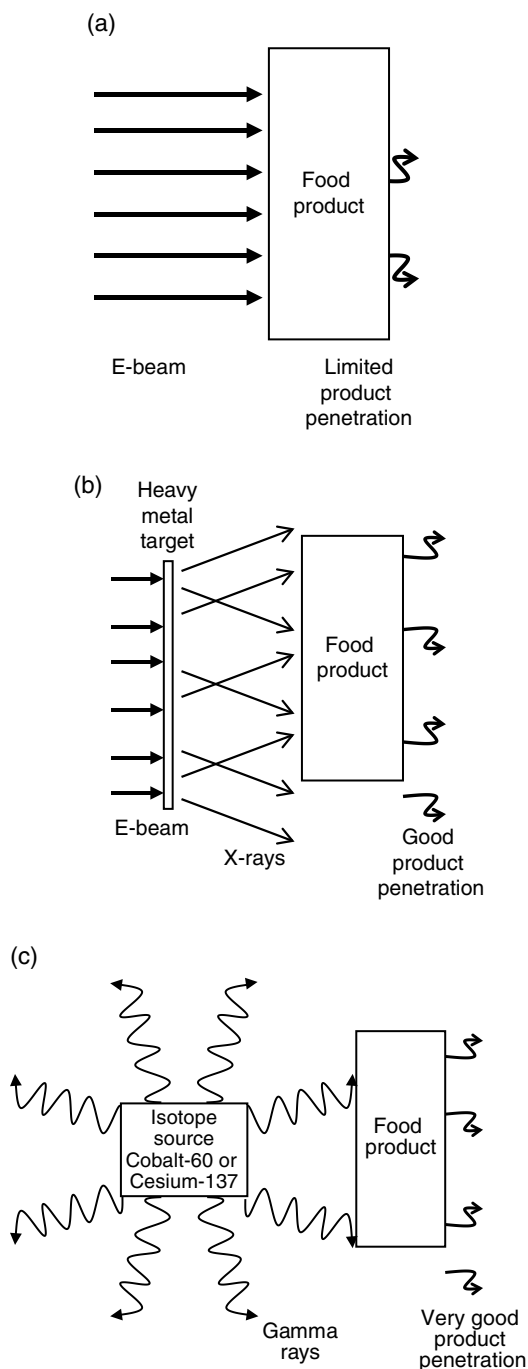
Figure 22.3 The three types of ionizing radiation utilized in the food industry: (a) e-beam generated by linear accelerator; (b) x-ray generated by e-beam striking a metal target; and (c) gamma radiation generated by a radioactive isotope emitting gamma rays.

likely be negligible; despite the minute temperature increase, e-beam processing is therefore considered a 'cold pasteurization/sterilization' process. This example also illustrates that if frozen foods were irradiated at sterilization doses (30–40 kGy), these frozen foods would experience a temperature increase of about 10°C (18°F) during irradiation and would be a consideration.

22.3.2 Comparison of e-beam, gamma radiation, and x-ray

The radiation source for x-rays, just like for an e-beam, is an electrical device called a linear accelerator that generates high-energy electrons. The x-ray is generated when a metal target is positioned between the e-beam and the food that is being targeted (Figure 22.3). The high-energy electrons generated from the linear accelerator collide with a metal target and in turn produce x-rays. X-rays penetrate better than e-beam; however, the e-beam is more energy efficient. For example, when an e-beam at an energy level of 5 MeV is converted to an x-ray, the resulting x-ray is about 10% of the incident e-beam power but has deeper penetration depth. Efficiency and product penetration depth should therefore be considered when making the choice between e-beam and x-ray radiation.

Gamma radiation has a similar penetration depth as x-ray. Unlike e-beam and x-ray, gamma radiation is not generated by a linear accelerator; it requires exposure of food to radioactive isotopes (i.e. Co-60 or Cs-137). These hazardous radioactive isotopes are always present at the processing facility and extreme care must be exercised to contain and dispose of the nuclear waste.

22.3.3 Mechanism of microbial inactivation

The mechanism of microbial inactivation by gamma radiation, x-ray, and e-beam generally occur in the same manner. With exposure to radiation, microbial growth is inhibited and death is usually accomplished when structural damage

(membrane breakdown, DNA conformational changes, protein aggregation, etc.) or physiological disorders (leakages from membrane, loss of key enzymes, etc.) occur. Since several of these maladies may occur simultaneously, it is difficult to attribute the loss of cell viability to a single event (Manas and Pagan, 2005). It is widely believed that the mechanism of inactivation for microorganisms by high-energy ionization radiation is due to two main mechanisms: direct interaction of the radiation with cell components and indirect action from free radicals generated by water radiolysis.

Direct effect reactions include interaction of high-speed electrons with cellular components such as chromosomal DNA, RNA, and microbial proteins. Each strand (helix) of the microbial DNA consists of four bases (G: guanine; C: cytosine; T: thymine; and A: adenine). The bases from separate DNA strands bond with each other, creating unique base pairs (G-C and T-A). The e-beam can break these G-C and T-A bonds, resulting in disruption of the double helix which renders microbial cells incapable of division, thereby 'killing' the organism by hindering reproduction (Figure 22.4). This is the main mechanism of direct microbial inactivation by e-beam.

The indirect effects of e-beam are due to cell surface damage and DNA damage as a result of bombardment of the cell membrane or DNA by free radicals (unstable molecules due to an extra electron) that are generated during water radiolysis (i.e. splitting of water molecules that are subjected to ionizing radiation) (Figure 22.5; Arena, 1971; Miller, 2005). The indirect effect of e-beam on microbial inactivation by cell surface damage is therefore dependent on water availability (i.e. water activity a_w) in a food product. For example, as photons of radiation penetrate a food product they encounter water molecules causing the *Compton Effect* where
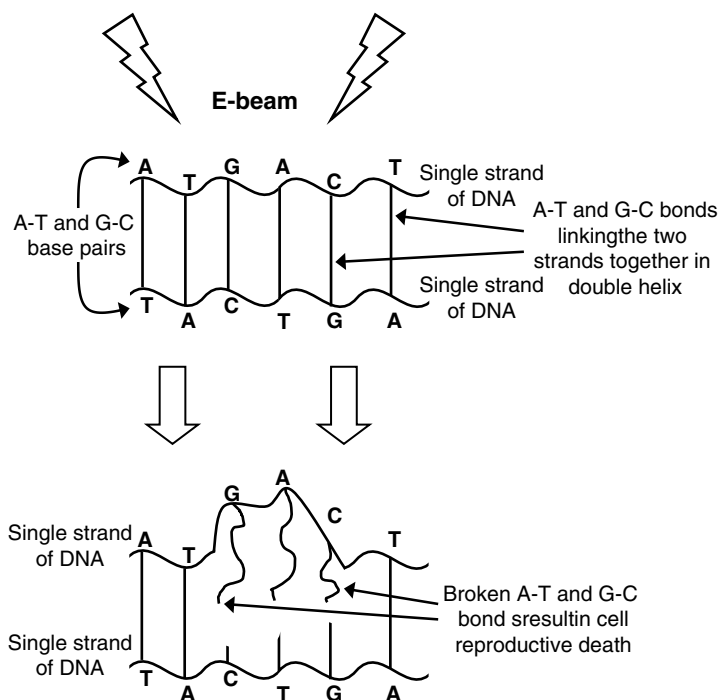
Figure 22.4 Direct effect of microbial inactivation by e-beam targets the genetic material (DNA and RNA) and breaks the base pairs G-C (guanine-cytosine) and T-A (thymine-adenine), resulting in reproductive death of microorganisms.
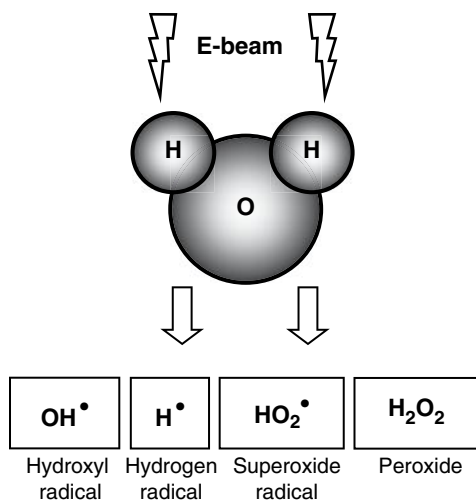
Figure 22.5   Indirect effect of microbial inactivation by e-beam is due to free radicals that compromise cell membrane. The free radicals are generated from water. Water molecules undergo radiolysis when subjected to e-beam yielding species with unpaired electrons (i.e. free radicals) (adapted from Arena, 1971).

the photons of radiation interact with water molecules, yielding a number of radiolytic products such as free radicals (Figure 22.6; Arena, 1971; Miller, 2005). Free radicals are extremely reactive and seek formation of stable products by combining with one another or with oxygen, thereby producing oxidizing agents. If these oxidizing agents and free radicals encounter a microbial cell, they will bombard and damage the cell membrane. If the injury is sufficient, cellular death will follow typically as a result of cellular leakage, and finally complete cell lysis. There is also an indirect effect of e-beam on microbial DNA due to the fact that 50–70% of bacterial cell mass is water. The incoming e-beam energy is therefore absorbed in the cytoplasm, resulting in the formation of hydroxyl radicals and hydrated low-energy electrons which also contribute to microbial DNA damage.
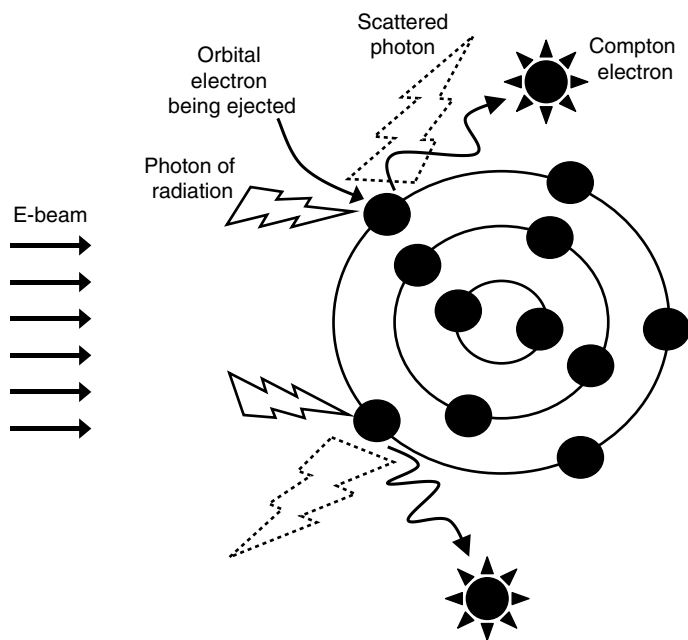
Figure 22.6   Incoming high-energy photons of radiation create the Compton effect, responsible for the increased absorption of e-beam dose under the surface of food products (up to 21 mm). Source: Tahergorabi *et al.*, 2012. Reproduced by permission from Elsevier.

22.4 Microbial inactivation kinetics and process calculations

The calculations to determine microbial inactivation by e-beam have been adapted from those used for thermal processing of food. When calculating microbial inactivation kinetics for e-beam-irradiated food, the first parameter that needs to be determined is the dose penetration by dose mapping. Once the dose at every location of a product is known, the D_{10}-value is used to calculate microbial inactivation in the product. The D_{10}-value is known as the 'decimal reduction' and is defined as the e-beam dose (kGy) that will induce a 1-log (i.e. 90%) reduction of a microbial population (Jaczynski and Park, 2003a,b, 2004; Figure 22.7). For irradiation processing, the subscript 10 is typically used to differentiate it from the D-value for thermal processing. The D-value subscript 10 for irradiation indicates the 'decimal reduction', while the D-value subscript for heat processing indicates the temperature (°C or °F) at which the D-value was determined. For example $D_{250°F} = 20$ seconds or $D_{10} = 0.25$ kGy indicates that 20 seconds at 250°F or 0.25 kGy, respectively, results in a 90% (1-log) microbial reduction. The D_{10}-values vary based on the target organism and medium; for example, the D_{10}-value for *Salmonella* in peanut butter would be different from the D_{10}-value for *L. monocytogenes* on cantaloupe.

The D_{10}-value is determined from survivor curves when the log of microbial population is plotted against e-beam dose (Figure 22.7). The D_{10}-value is an inverse reciprocal of a slope of the survivor curve. Mathematically, the survivor curve is represented by Equation (22.1). The D_{10}-value can therefore be calculated from
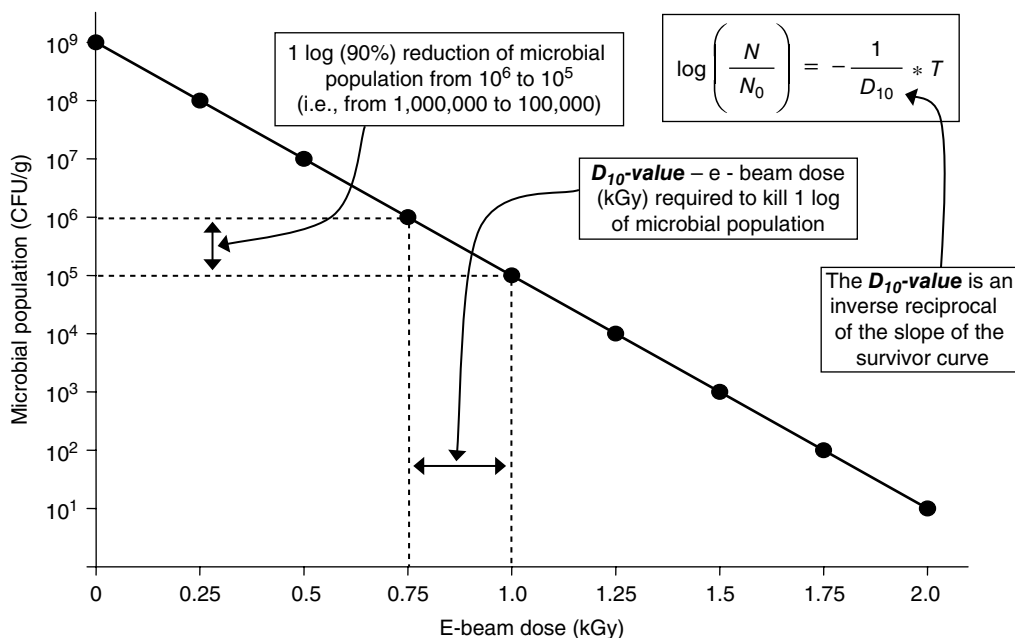
Figure 22.7 The microbial survivor curve. The D_{10}-value is the e-beam dose (kGy) required to kill 1-log (90%) of a microbial population of a given microorganism in a specific food. The D_{10}-value can be calculated as an inverse reciprocal of the slope of the survivor curve.

Equation (22.2) (Singh and Heldman, 2003; Su *et al.*, 2005):

$$\log\left(\frac{N}{N_0}\right) = -\frac{1}{D_{10}}T \qquad (22.1)$$

$$D_{10} = \frac{T}{\log(N_0) - \log(N)} \qquad (22.2)$$

where T is the e-beam dose (kGy); N_0 is the initial microbial population; and N is the final microbial population surviving the T e-beam dose.

22.5   Microbial radio-resistance

Microbial adaptation under conditions that were previously thought to inhibit microbial growth, i.e. low pH, high temperature, antibiotics, etc., leads to the development of microbial resistance to the environmental stressor. Since some types of bacteria are more adaptable to adverse environmental conditions, subsequent populations of survivors become more resistant. For example, when certain antibiotics were applied to treat *Staphylococcus aureus* infection, cells that were naturally resistant to these drugs were able to survive treatment and were inadvertently selected for their resistance. This is a simplified example of the evolution of methicillin-resistant *S. aureus* (MRSA). When it comes to radio-resistance, the ability of a bacterium to efficiently repair radiation-induced damage of their DNA is directly related to their ability to build resistance (Venugopal and Bongirwar, 2002). Levanduski and Jaczynski (2008) demonstrated that when e-beam was repetitively applied to *Escherichia coli* O157:H7 in ground beef, recovered cells developed increased radio-resistance. Tesfai *et al.* (2011a, 2011b) also investigated the effect of repeated, sublethal e-beam exposure on the microbial radio-resistance of *S.* Typhimurium in egg and DNA repair-deficient *E. coli* DH5 α in ground beef. E-beam efficiently inactivated *Salmonella* in raw egg and *E. coli* DH5α in ground beef; however, similar to other inactivation techniques and factors affecting microbial growth, *Salmonella* and *E. coli* DH5α developed increased radio-resistance after repetitive sublethal doses (Tesfai *et al.*, 2011a, b).

22.6   Foodborne *Salmonella* outbreaks and *Salmonella* reservoirs

Contamination with *Salmonella* can occur at any stage of the production chain such as during growth, harvesting, processing, distribution, and final preparation. The vehicles of pre-harvest contamination may include birds and other animals such as insects; pathogenic organisms may also be present in the water or soil, or introduced by dirty equipment and improper hygiene by human handlers. However, a consistent source of contamination is likely to be the application of manure or compost as fertilizer to fields where crops are grown and the fecal contamination of irrigation water. Cattle are a transient reservoir of pathogens; typically they are asymptomatic carriers and shed the pathogens in their feces. Pathogenic organisms are able to survive in manure for extended periods (Wang *et al.*, 1996; Kudva *et al.*, 1998; Bolton *et al.*, 1999; Scott *et al.*, 2006) and manure-amended soil (Mubiru *et al.*, 2000; Gagliardi and Karns, 2002; Ogden *et al.*, 2002; Franz *et al.*, 2008; Franz and Van Bruggen, 2008). *Salmonella* and *E. coli* will contaminate the surface of vegetables when grown in soils enriched with contaminated manure (Natvig *et al.*, 2002; Islam *et al.*, 2004a, b). When surface-sterilized leaf tissue of lettuce seedlings grown in manure-amended soil were examined, high concentrations of *S.* Typhimurium were recovered (1.10^2–1.10^4 CFU g^{-1}; Franz *et al.*, 2007; Klerks *et al.*, 2007). Pathogenic bacteria such as *Salmonella* and *E. coli* can also internalize into the edible parts of different varieties of plants like tomato (Guo *et al.*, 2002), radish sprouts (Itoh *et al.*, 1998), bean sprouts (Warriner *et al.*, 2003), barley (Kutter *et al.*, 2006), and lettuce (Solomon *et al.*, 2002; Wachtel *et al.*, 2002; Franz *et al.*, 2007; Klerks *et al.*, 2007).

Contamination of eggs with *Salmonella* not only occurs on the surface of the shell, but also by the passage of the organism from the intestinal tract of the hen to the reproductive system where it is incorporated into the developing egg (Humphrey, 2004). Different serotypes of *Salmonella* can pass from the intestine of the hen into the blood stream and then to the reproductive tract. However, unlike other serotypes, *S*. Enteritidis has a unique capability to survive in the oviduct of the hen. The exact reason for the epidemiological association of *S*. Enteritidis with eggs is still undefined (Gantois *et al*., 2009).

Mild heat pasteurization of liquid whole egg and egg yolk has become well established in the past decade. This type of thermal pasteurization cannot be applied to whole eggs still in the shell because the heat would simply cook the egg. Since there is minimal heat transfer with e-beam irradiation, there is potential for this technology for the control of *Salmonella* in and on whole in-shell eggs.

Seafood is also at risk of contamination by *Salmonella*, especially if it has been harvested from contaminated bodies of water. There have been documented seafood-related outbreaks of *Salmonella* in the European Union (EFSA, 2011), the United States (CSPI, 2009) and other countries worldwide. The US Food and Drug Administration (FDA) has identified the presence of *Salmonella* in a variety of fish and shellfish, including ready-to-eat (RTE) seafood products, seafood products requiring minimal cooking, and shellfish eaten raw (Heintz *et al*., 2000; Brands *et al*., 2005; Duran and Marshall, 2005). Most often, seafood is exposed to *Salmonella* from polluted waters; however, contamination may also occur during storage and processing (Panisello *et al*., 2000). Seafood that falls into the highest risk category for inducing FB illness includes mollusks (fresh and frozen mussels, clams, oysters) and fish that are served raw (Huss *et al*., 2000). Seafood that is intended to be eaten after cooking does not pose as great a risk; however, cross-contamination is possible from contaminated fish to other products. Lightly preserved fish products (salted, marinated, fermented, cold smoked, and gravad fish), semipreserved fish (caviar), mildly heat-processed (pasteurized, hot-smoked), and heat-processed (sterilized, packed in sealed containers) pose the lowest risk of illness by *Salmonella*; however, post-processing contamination is still possible.

While *Salmonella* has been implicated as the cause of FB illness in raw animal food products such as meat, poultry, milk, and egg products, other recent outbreaks have been associated with peanut butter, cucumbers, mangoes, and cantaloupe (CDC, 2013). In November 2006, *S*. Tennessee was the causative agent of a multistate outbreak related to the consumption of peanut butter; however, the source of contamination was unknown (CDC, 2013). An outbreak of *S*. Typhimurium in peanut butter that followed that original outbreak was traced back to a manufacturing plant in Georgia (US). This multistate outbreak sickened 714 people and possibly contributed to 9 deaths (CDC, 2013). Since peanut butter is commonly used as an ingredient in many different food products, the fall-out of the contamination resulted in many peripheral recalls including products such as cookies, crackers, cereal, candy, ice cream, and pet foods, all of which originated from the Georgia facility (Maki, 2009). Although CDC and FDA warned consumers to refrain from consumption of the recalled products, more outbreak-related illnesses continued to be reported for the several months after, partly because many of the implicated products had already been widely distributed. More recently, in 2012 peanut butter was linked to an outbreak of *S*. Bredeney that sickened 42 people, with over half of those sickened under the age of 10 (CDC, 2013).

Outbreaks associated with produce occur throughout the year, with most FB illnesses from fresh fruits and vegetables peaking in the spring and summer and tapering in the fall (Franz and Van Bruggen, 2008). The food items most frequently implicated in produce-associated outbreaks include vegetable salads, lettuce, various fruit juices, melon, and sprouts (Sivapalasingam *et al*., 2004). Although various pathogens have been implicated in produce-related outbreaks, *Salmonella* is the most

common. Tomatoes in particular have been associated with several multi-state outbreaks of *Salmonella* in the last decade. Three salmonellosis outbreaks associated with consumption of Roma tomatoes occurred in the summer 2004. During this period, 561 confirmed salmonellosis cases were associated with tomatoes from at least one packinghouse in the US, raising concerns over industry washing and decontamination practices. Major outbreaks of *S*. Saintpaul continue to occur in fresh produce, with cucumbers being the most recent (2013) vehicle of transmission, followed by alfalfa sprouts (2009), and jalapeño peppers (2008) (CDC, 2013). Cantaloupe was the vehicle for a 2012 *Salmonella* outbreak that sickened 261 people and resulted in 3 deaths (CDC, 2013).

22.6.1   Examples of e-beam applications to inactivate *Salmonella* in food

Traditionally the food industry has depended on the acidity of certain food products to protect against the growth of *Salmonella*; however this reliance has been challenged by the recent salmonellosis outbreaks associated with acidic food products, including several outbreaks associated with tomatoes (Hedberg *et al.*, 1999; Sivapalasingam *et al.*, 2004; CDC, 2013). James *et al.* (2010) reported significant reductions of nalidixic acid-resistant *S*. Montevideo in pureed tomatoes when exposed to e-beam doses ≤1.5 kGy. The D_{10}-value for this organism in tomato puree was relatively high (average 1.28 kGy); it is possible that the antioxidant properties of the medium may have provided protection against the e-beam radiation. On the other hand, when fresh-cut tomatoes and spinach were exposed to e-beam, rifampin-resistant *S*. Montevideo and *S*. Agona were significantly reduced at lower doses (<1.0 kGy) (Schmidt *et al.*, 2006; Neal *et al.*, 2008). At these doses, no product damage as a result of e-beam radiation was reported.

Prior to packaging, peanut butter undergoes heat treatment at 70–75 °C; it has been reported that *Salmonella* survives in peanut butter at 90 °C for 45 min (Shachar and Yaron, 2006). If the temperature or holding time is increased to over 90 °C for 45 min, it is very likely that flavor, texture, and overall quality would be impacted. Due to the recent and highly publicized outbreaks of salmonellosis associated with peanut butter, Hvizdzak *et al.* (2010) showed that e-beam effectively reduced *S*. Tennessee and *S*. Typhimurium in peanut butter at 3.0 kGy e-beam dose. It is likely that cells were more resistant to e-beam radiation in peanut butter because peanut butter has a low water activity (a_w). Matak *et al.* (2010) demonstrated that e-beam could cause injury to *S*. Tennessee and *S*. Typhimurium; however, cells were not able to convalesce in in the high-fat, low-a_w peanut butter environment. This demonstrates the need for an effective strategy to control *Salmonella* in peanut butter; once *Salmonella* is introduced into a low-a_w food like peanut butter, it survives for the duration of the intended shelf-life (Burnett *et al.*, 2000).

22.7   US regulatory status of e-beam

Since e-beam inactivates harmful microorganisms by generating ionizing radiation it falls into the same legal category as gamma and x-ray radiations. The US Code of Federal Regulations (CFR; 21 CFR 179.25) describes the general provisions for food irradiation. According to the Code, food treatment by radiation must be conducted according to a standard written procedure developed by a qualified person with expert knowledge in radiation processing requirements specific to that food. This protocol includes the radiation dose range that is adequate to achieve its intended effect (i.e. delay of sprouting, pest, and microbial disinfection, etc.) on the specific food product. Treatment records (including food treated, lot identification, scheduled process, evidence of compliance with the scheduled process, ionizing energy source, source calibration, dosimetry, dose distribution in the product, and the date of irradiation) must be maintained and available for inspection by FDA for 1 year

past the product shelf-life or 3 years, whichever period is shorter (21 CFR 179.25).

Irradiated foods must be labeled with the radura sign (Figure 22.8) and be accompanied by the words 'treated with radiation' or 'treated by irradiation'. This statement does not need to indicate the purpose of food irradiation (i.e. to control *Vibrio* spp. in shellfish or *Trichinella spiralis* in pork); however, when consumers are informed that the radiation kills harmful bacteria and does not make food radioactive, they may be more likely to purchase irradiated foods. A consumer survey showed that 80% of respondents ($n = 1003$) would be 'somewhat likely' or 'very likely' to buy irradiated foods for themselves or their children if the required statement was revised to say 'irradiated to kill harmful bacteria' (Murano, 2003).

When irradiated food is sold in bulk or not in a package, the radura logo and phrase are to be displayed in plain view or each item is to be individually labeled. However, if the food is used as an ingredient in another food product, the radura logo and phrase must accompany the ingredient to the purchaser, but does not need to be on the label of the final product. For example, if irradiated spices are used as an ingredient in a food, no label is required as long as the entire product has not been treated with radiation. If irradiated foods are available in restaurants, no point-of-purchase labeling is required. For example, if

hamburger patties are irradiated, the restaurant selling them does not have to indicate on the menu that they have been treated with radiation.

The safety of consumption of the irradiated foods, including foods processed with e-beam, is well established; however, the CFR has set limitations on the maximum allowable dose of radiation depending on type of food and intended use of treatment (i.e. to delay sprouting, pest and microbial disinfection, etc.; Table 22.1). Wheat and flour were the first foods to be approved by FDA in 1963 to control insects, followed by white potatoes in 1964 to inhibit sprouting (EPA, 2012). Pork was the first fresh meat to be approved in 1985 for the elimination of *Trichinella spiralis*, the causative agent of trichinosis (EPA, 2012; Table 22.1). The approval for pork irradiation paved the way for consent of several vegetable and fruit products for inhibiting growth and maturation, and also for disinfection of pests. Food irradiation is now approved for pathogen reduction and sterilization at doses not less than 44 kGy.

Table 22.1 Some examples of products approved for food irradiation in the United States

Petition	Purpose	Max. dose (kGy)
Fresh vegetables	Disinfestation	1
Fruit	Disinfestation	1
Pork	Decontamination	1
Teleost fish and fish products	Decontamination	2.2
Poultry (fresh or frozen)	Decontamination	3
Shell eggs	Decontamination	3
Fresh red meat	Decontamination	4.5
Fresh and frozen molluscan shellfish	Decontamination	5.5
Frozen red meat	Decontamination	7
Dry vegetable seasonings	Decontamination	30
Herbs	Decontamination	30
Spices	Decontamination	30
Frozen meats for NASA space programs	Sterilization	Minimum 44

Figure 22.8   The radura sign and the statement 'treated with radiation' or 'treated by irradiation' must be displayed on the package of e-beam processed food products.

The process for FDA approval for irradiation takes a long time. As an example, a petition for the irradiation of fresh and frozen molluscan shellfish (oysters, mussels, clams, etc.) was submitted by the National Fisheries Institute (NFI) and the Louisiana Department of Agriculture and Forestry (LDAF) to the FDA in 1999 (Food Additive Petition FAP 9 M4682) (Federal Register, 2005). According to the Federal Food, Drug, and Cosmetic Act (12 USC 321(s)) section 201(s), the source of radiation used to process food is considered to be a food additive; the NFI and LDAF were therefore responsible for addressing the potential toxicity, nutritional adequacy, and microbiological risks of consuming the irradiated food. If the necessary studies had not already been conducted, then it was up to them to demonstrate the safety of the 'additive'. Ultimately, it took until August 2005 for the FDA to approve irradiation of frozen and fresh molluscan shellfish at ≤5.5 kGy to control *Vibrio* spp. and other foodborne pathogens (21 CFR Part 179 Docket No. 1999F–4372; Federal Register, 2005).

Widespread commercialization and adaptation of food irradiation has stalled; reasons for this delay include anti-food irradiation activism, the time-consuming approval process, and lack of consumer knowledge of the benefits and risks (Griffith, 1992; Forsythe, 2000). The future of irradiated foods ultimately rests in the hands of the consumers and the key is through increased consumer education efforts.

22.8   Future direction of *Salmonella* inactivation using e-beam

The food safety benefits of e-beam are well established; however, high-energy continuous e-beam in food processing is limited due to the large size of the unit, voltage requirements, complex shielding requirements, and safety of installation. Improvements in e-beam technology have therefore focused on the development of a compact, low-energy, high-power, pulsed e-beam that has the potential to be developed into a household device that integrates e-beam with a microwave oven. This unit would be capable of operating in two modes: microwave or e-beam. The microwave mode would continue to work just like a normal microwave, that is, by applying non-ionizing microwave radiation to foods that result in heating/cooking as the energy is absorbed. The e-beam mode would be a non-thermal alternative effective at reducing microbial pathogens without cooking 'fresh' food products such as vegetables and others. This unit would be the first antimicrobial strategy at the point-of-consumption (i.e. household and food service level) capable of inactivating foodborne pathogens in a non-thermal manner. The secondary emission electron gun (SEEG) is a novel microbial intervention that is much more compact than a traditional e-beam unit. Combining this technology with microwave technology is of interest.

The SEEG produces a low-energy (<100 keV), high-power (180 KW), and short duration (5 μs) pulsed electron beam for non-thermal surface irradiation of food products (Chalise *et al.*, 2001, 2004; Figure 22.9). The technology (SEEG) uses a unique accelerator design that was developed by scientists at Tokyo Institute of Technology. When in operation, helium ions are generated at a thin wire plasma discharge device referred to as Wire Ion Plasma Source (WIPS). The energetic helium ions are accelerated in a vacuum towards a negatively charged cold cathode and a large number of secondary electrons are generated through a kinetic emission process. The secondary electrons emitted from the cathode surface are accelerated towards the electron window, where they form a wide and uniform electron beam. The electron window is the partition between the atmospheric air decontamination chamber and the SEEG that is inside the vacuum chamber. The e-beam transmitted through the window is uniform when it enters the decontamination chamber where it encounters the food (Chalise *et al.*, 2001).

The SEEG e-beam is effective at reducing non-pathogenic *E. coli* (Chalise *et al.*, 2004, 2007) and *Bacillus subtilis* in vegetative and spore forms
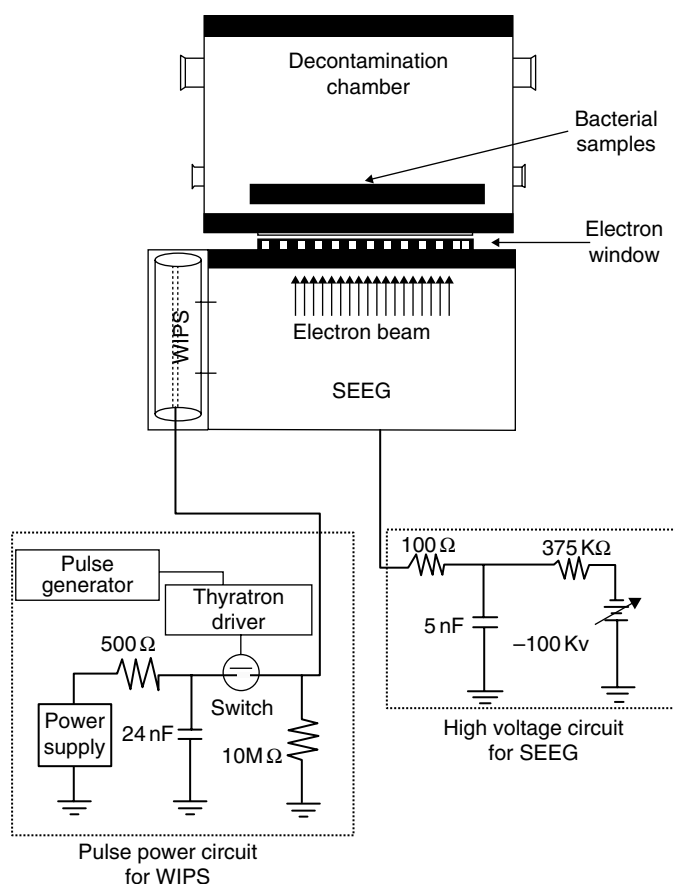
Figure 22.9   A diagram of experimental secondary emission electron gun (SEEG) e-beam including decontamination chamber, wire ion plasma source, secondary emission electron gun, and power systems. Chalise *et al.*, 2007. Reproduced with permission of Wiley.

(Rahman *et al.*, 2006). However, the SEEG e-beam has not yet been tested for its efficacy against typical foodborne pathogens in different food matrices and it is likely that penetration of the electrons into the product will continue to be an issue.

22.9   Conclusions

Salmonella continues to be a major causative agent of foodborne illness worldwide. While thermal pasteurization is well established and adequately reduces *Salmonella* in many food products, it is not suitable for fresh food products such as fruits, vegetables, fresh shell eggs, etc.

E-beam irradiation is effective at reducing *Salmonella* as well as other microorganisms, viruses, and insects in foods. The main benefits of this technology include the anti-microbial effect, a minimal rise in temperature during processing (cold-pasteurization), and it does not require the use of radioactive isotopes to generate radiation. Consumer safety of irradiated foods is well established and it is approved by the Food and Drug Administration for use on meat, poultry, spices, fresh fruits, and vegetables. It is likely that application of electron beam in the food processing industry will expand as consumer knowledge and acceptance of the technology grows.

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23

Inactivation of Foodborne Viruses: Recent Findings Applicable to Food-Processing Technologies

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Summary

Foodborne viruses such as noroviruses have become a huge food safety concern, being responsible for more than half of all foodborne illnesses. The high prevalence of foodborne viruses in the food sector may be related to several factors such as their high persistence in the environment and in foods, as well as their low infectious dose. Even if a treatment reduces an initial viral load of 5 $\log_{10}$ by 1000-fold, residual unaffected virions (0.01%) may be sufficient to cause illness. Minimally processed foods such as bivalve molluscs and fresh produce are frequently involved in the transmission of foodborne viruses to humans. This chapter provides an update of the recent findings relating to the inactivation of foodborne viruses using physical and chemical approaches. In addition to traditional methods using heat or chemical compounds such as chlorine and organic acids, innovative technologies such as high-pressure, pulsed light and irradiation as well as ozone or peroxyacid treatments are presented.

23.1   Introduction

Food safety is of great concern in both the food and health sectors. The World Health Organization (WHO) defines a foodborne illness as a disease caused by infectious agents transmitted to humans through ingestion of contaminated foods (WHO, 2007). The number of incidences is increasing year by year. In the United States for example, among the 216 million cases of infectious illnesses reported each year, 9.4 million are caused by foodborne agents (Scallan *et al.*, 2011a). These are primarily viruses (59%), followed by bacteria (39%) and parasites (2%). Among the viruses, noroviruses cause over 5 million cases of gastroenteritis per year (more than 99% of viral foodborne illness). Sapoviruses, rotaviruses, astroviruses and, to a lesser extent, hepatitis A virus (HAV) are other significant causes of gastro-enteric illness (Scallan *et al.*, 2011b). Adenoviruses, hepatitis E virus, aichivirus and enteroviruses such as poliovirus are sometimes implicated. In general, enteric viruses are non-enveloped RNA viruses that invade the human body via the intestine, are excreted in human feces and are transmitted by the faecal–oral route. Foodborne viruses have been the subject of a review by Greening (2006).

Although it has long been known that viruses may be transmitted by foods, the first evidence of this was reported in 1915 when raw milk was associated with the transmission of poliomyelitis to humans. The incidence of foodborne viral illness was underestimated until the 1990s when molecular tools were developed. Viral contamination of foods can occur pre-harvest or at any stage in the harvesting, processing, distribution and preparation of foods. The increasing consumption of raw foods and the globalization of international trade have contributed to the increase in the number of outbreaks of viral foodborne illness (Gerba, 2006). Shellfish, berries, herbs and salads are the foods at the greatest risk of contamination by viruses at the pre-harvest stage. Foods requiring more preparation and subsequently consumed raw, cold or slightly cooked are at increased risk of contamination by food handlers. These include notably bread and

bakery goods, sandwiches or cold desserts (Richards, 2001). Current food hygiene guidelines, which are optimized for the prevention of bacterial growth, may be insufficient against viruses (Koopmans and Duizer, 2004).

Controlling foodborne viruses in the food sector represents a daunting challenge compared to bacteria and fungi for several reasons. Their presence in foods is not detectable without sophisticated means, since they are inert particles that do not replicate in the absence of specific living host cells. In addition, the ingestion of only a few virions may be sufficient to cause illness unlike bacteria, which in most cases do not cause illness unless thousands of live cells are ingested. Finally, viruses withstand a wide variety of commonly used food processing and preservation treatments and storage conditions, unlike vegetative bacterial and fungal cells. They are also able to withstand the extreme acidic conditions and enzymatic attacks of the host gastrointestinal tract (Gerba, 2006). Viruses can persist for long periods on surfaces and in foods until they meet their host cells and cause infection (Greening, 2006). The three principal mechanisms of inactivation of viruses are depicted in Figure 23.1.

Several techniques have been developed in order to reduce microbial growth and metabolism in foods. Nicholas Appert in 1809 invented canning as a food preservation technique, a half-century before the existence and significance of microbial life in foods were explained by Louis Pasteur (Ngadi *et al.*, 2012). Although thermal processing is suitable for reducing the numbers of viable microbial cells in many food matrices, it is not applicable to fresh produce which is eaten raw for enjoyment of texture and flavour. Heating may alter the tissue structure and flavour of fruits and vegetables beyond recognition, as well as decreasing the vitamin content. Various non-thermal processing techniques collectively known as cold pasteurization (Hogan *et al.*, 2005) have been developed in addition to the use of chemical additives. These must ideally be active against a wide range of pathogenic organisms, easy to use, economical and must not result in the production of toxic or dangerous byproducts
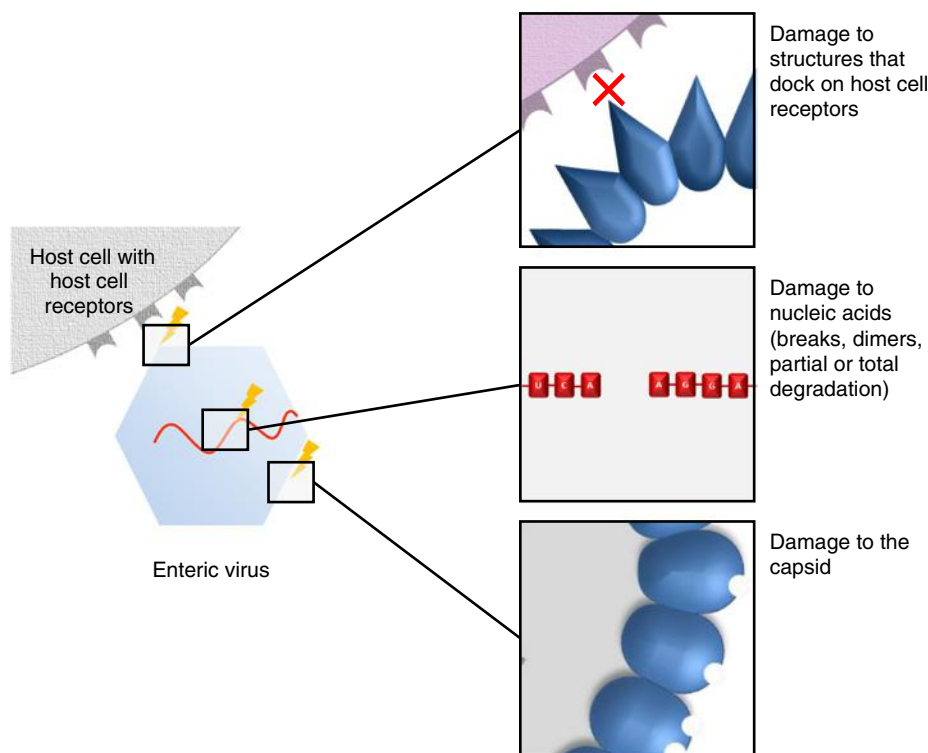
Figure 23.1 Overview of inactivation mechanisms of viruses. For color details, see color plates section.

(Rudd and Hopkinson, 1989). A treatment that reduces the infectious viral particle titre by 3 or 4 $\log_{10}$ cycles is generally regarded as satisfactory (Sattar and Bidawid, 2006).

In this chapter, an overview of the antiviral effects of traditional and more recent methods used in the food sector is presented. These treatment methods, summarized in Table 23.1, are divided into two major groups, namely physical (Sections 23.2) and chemical (Sections 23.3).

23.2 Physical treatments

23.2.1 Low-temperature-based methods

Low (and high; see following section) temperatures are the most widely used methods of preservation in the food industry. These methods are based on the exchange of heat, defined as

the form of energy that can be transferred from one medium to another across a temperature gradient.

Low temperatures refer to freezing (below $-10\,°C$) and refrigeration (between -1.5 and $+8\,°C$), which increase the shelf life of the product for a limited period by slowing enzymatic reaction rates and microbial growth. However, viral persistence might be slightly altered at low temperature, especially below $10\,°C$ (Lamhoujeb *et al.*, 2009; Bertrand *et al.*, 2012) as well as at room temperature (Dawson *et al.*, 2005), regardless of the matrix (food, water, inert surface). For instance, HAV, norovirus and rotavirus remain infectious on various frozen fruits (at $-20\,°C$) for up to 90 days, with reductions of less than 1 $\log_{10}$ (Butot *et al.*, 2008). These observations corroborate data from documented outbreaks involving frozen fruits contaminated by HAV or norovirus (Hjertqvist *et al.*, 2006).

Table 23.1   Overview of physical and chemical treatments used to inactivate foodborne viruses

	Treatment	Solid foods	Liquid foods	Food contact surfaces	Advantages	Disadvantages
PHYSICAL TREATMENTS	High temperature	Y	Y		Commonly used	Not applicable to all food products
	UV light	Y	Y	Y	Easy to use	Oxidation, flavour changes; uses mercury (toxic vapour)
	Pulsed light	Y	Y	Y	Easy to use; no toxic compounds generated; no food quality changes	Thermal effects (food surface)
	Irradiation	Y			Applicable to frozen foods and packaged foods	Negative consumer opinion
	High pressure	Y	Y		Applicable at room temperature; minimal change in food quality	Huge capital cost
CHEMICAL TREATMENTS	Hypochlorous acid	Y	Y	Y	Very effective in certain situations	Toxic byproducts
	Chlorine dioxide	Y	Y	Y	No toxic byproducts	Explosive gas
	Ozone	Y		Y	No toxic byproducts	Toxic gas
	Peracetic acid	Y		Y	No toxic byproducts	Reactive with certain materials

23.2.2   High-temperature-based methods

23.2.2.1   Principle and mode of action

High-temperature treatments, especially pasteurization and sterilization, have long been a major processing technology in the food industry because of their significant destructive effect on microbial life. Heat can be transferred in three ways: by convection, conduction or radiation. Recent advances have focused primarily on conduction (also called ohmic heating) and on radiation (microwaves and radiofrequency waves) as heat transfer mechanisms, while convection is regarded as conventional (Ngadi *et al.*, 2012).

Heat processing is safe, chemical-free and produces tenderness and cooked flavours while conferring long food shelf life by eliminating most, if not practically all, spoilage organisms. However, many foods involved in outbreaks of viral illness are fresh produce intended for consumption without heat treatment, in order to provide the enjoyment of crunchy texture and fresh (uncooked) flavour as well as the benefit of full nutritional value (Rahman, 2007a). In order to preserve the market value of these products, novel non-thermal technologies such as UV and pulsed light, irradiation and high-pressure processing are being studied as alternative means of inactivating viruses in foods.

Temperatures higher than 65 °C inflict damage on surface molecules such as receptors by causing irreversible modification of protein secondary, tertiary and quaternary structures (Volkin *et al.*, 1997; Ausar *et al.*, 2006). Viruses subjected to such conditions lose their infectious capability, while their nucleic acids remain detectable by molecular methods. This implies that high temperatures do not destroy nucleic acids and that, although the viral capsid is damaged by heat, it continues to protect the nucleic acid against the surrounding physical conditions (Nuanualsuwan and Cliver, 2003; Baert *et al.*, 2008). In practice, no correlation between viral infectivity and detection of RNA

can be established. Molecular methods are therefore suitable for establishing whether or not a heat-treated food is or has been contaminated by a virus, but do not indicate whether or not it is safe to consume (Croci *et al.*, 2012).

23.2.2.2 Virus inactivation by high-temperature treatments and factors affecting their effectiveness

Inactivation of viruses is generally much more extensive at higher processing temperatures. Many studies have focused on the effects of light cooking on the inactivation of enteric viruses in shellfish, since these foods are often involved in outbreaks of viral illness (Mattison, 2011). These studies show that an internal temperature of 70 °C is insufficient to produce significant inactivation (Hewitt and Greening, 2004; Croci *et al.*, 2005; Harlow *et al.*, 2011). It is generally assumed that a few minutes at a temperature close to 100 °C are necessary in order to achieve more than a 3 $\log_{10}$ reduction of viral titre in foods (Deboosere *et al.*, 2004; Baert *et al.*, 2008; Hewitt *et al.*, 2009; Sow *et al.*, 2011) or on inert surfaces (Eterpi *et al.*, 2009). Somatic and F-specific bacteriophages have been proposed as model viruses for high-temperature treatment 'worst-case scenarios', while HAV seems to be less resistant at temperatures above 50 °C (Bertrand *et al.*, 2012).

In complex food matrices, prolonged treatments at high temperatures are needed in order to achieve significant inactivation of viruses (Bertrand *et al.*, 2012). Heat treatments can even cause the formation of viral aggregates depending on the type of food, the ionic composition of the medium, the pH and the isoelectric point of the virus (Floyd and Sharp, 1979). Protein, fat and sugar protect virions against heat (Bidawid *et al.*, 2000b; Hewitt *et al.*, 2009). For instance, whereas complete inactivation of HAV (i.e. 5 $\log_{10}$ reduction) in shellfish meat required steaming to an internal temperature of 85–90 °C for 1.5 min (Millard *et al.*, 1987), 30 seconds at 75 °C were sufficient to achieve a 6 $\log_{10}$ reduction of this virus suspended in buffer (Parry and Mortimer, 1984). It has also been observed that inactivation is faster at lower pH (Deboosere *et al.*, 2004; Seo *et al.*, 2012).

23.2.3 UV light treatments

Light is a form of electromagnetic radiation, and as such transports energy from one location to another. This energy is carried by mass-less units called photons, each with a specific frequency and wavelength and associated electric and magnetic field components (Bolton and Cotton, 2008). The word *light* can refer to visible light or more generally to radiation at any wavelength from 100 to 1000 nm, which includes visible light and its adjacent spectral regions, namely ultraviolet (UV) light and near infrared (IR) radiation. In this chapter, light refers to this broader range of wavelengths, also called photochemical.

The quantity of light reaching the target is expressed as irradiance in watts per square meter (W m^{-2}) or as fluence (W s m^{-2} = J m^{-2}), which is irradiance multiplied by the exposure time (Bolton and Linden, 2003). Proper calculation of the fluence is the most important factor in evaluating a light treatment since it allows direct comparisons of different treatments, regardless of the experimental setup. It is nevertheless sometimes improperly reported.

23.2.3.1 Principle and mode of action

UV-C (wavelengths from 200 to 280 nm) treatment of preserved foods was discovered in the 1930s. UV light is emitted by a mercury lamp in a continuous mode, contrary to pulsed light (Artès and Allende, 2005). The emitted spectrum can be monochromatic (253.7 nm) or polychromatic (from 250 to 600 nm) depending on the vapour pressure of the mercury (Koutchma *et al.*, 2009b, 2009d). UV light is now used in a wide variety of industrial fields and in particular for the treatment of solid foods, liquid foods and potable water (Djenane *et al.*, 2001; Whitby and Scheible, 2004; Koutchma *et al.*, 2009c), applications now approved by the US Food and Drug Administration (FDA, 2011f).

UV light is a minimal process with many benefits including low cost, easy handling and use, and

lack of irritating or toxic byproducts (Hirneisen *et al.*, 2010). Furthermore, unlike chlorine, UV light does not cause any changes in the colour, flavour, odour or pH of water (Bintsis *et al.*, 2000). However, UV light is strongly absorbed by most of materials and cannot penetrate beyond the surface layer of solid objects (Koutchma *et al.*, 2009a). For this reason, inactivation of viruses by UV light is subject to physical constraints and limited primarily to highly transparent materials and non-reflective surfaces (Palmieri and Cacace, 2005). In addition, UV light may cause quality defects such as oxidation of fats, resulting in off-flavours such as rancidity, fishiness, 'cardboard' flavour and oxidized flavour. Retinoids, a class of chemical compounds related to vitamin A, are also very susceptible to oxidation because of their alkyl chains with highly conjugated double bonds (Rahman, 2007c).

Data available for bacterial and eukaryotic cells, combined with those obtained for the inactivation of viruses, have contributed to a consensus regarding the mode of action of UV light. Nucleic acids are the principal site of the damage inflicted by UV, which induces photoproduct formation, mostly cyclobutane dimmers of adjacent pyrimidine bases (Bintsis *et al.*, 2000). At low levels, these photoproducts are responsible for mutations since they interfere with normal base pairing, while beyond a certain level the organism is too damaged to remain viable. Viruses exposed to UV light are unable to replicate and are thus inactivated (Koutchma *et al.*, 2009b). To a lesser extent, UV light affects capsid proteins, leading to distortion of the capsid (De Sena and Jarvis, 1981), exposing the genome to environmental ribonucleases (or at least causing a loss of the ability to dock on and enter the host cell).

23.2.3.2   Virus inactivation by UV light treatments

Most of the reported studies have been conducted with suspensions of virus in water or buffer. These studies suggest that adenoviruses are the most resistant to UV light, while viruses of the family *Microviridae* seem to be the least resistant (Gerba *et al.*, 2002; Nuanualsuwan *et al.*,

2002; Thurston-Enriquez *et al.*, 2003). There are very few investigations of the inactivation of viruses on foods by UV light. Fino and Kniel (2008) evaluated the effect of UV light (120 mJ cm^{-2}) on feline calicivirus, aichivirus and HAV on the surface of lettuce, greens onions and strawberries. Reductions greater than 3 log$_{10}$ were observed on lettuce and green onions but not on strawberries, regardless of the virus tested.

23.2.3.3   Factors affecting the effectiveness of light treatments

Many factors may affect the efficacy of light treatments including UV and pulsed-light (see following section). These are divided into two groups: those affecting the light path and those associated with viral characteristics. The most important physical factor is the fluence reaching the viruses in the sample. Anything that interferes with the fluence will decrease inactivation. Fluence may be affected by treatment parameters such as the amount of energy delivered (Roberts and Hope, 2003), treatment duration or number of pulses (Wekhof *et al.*, 2001), or the distance from the light source to the target (Jean *et al.*, 2011). Parameters associated with the sample, such as the presence of UV absorbers (Koutchma *et al.*, 2009a), the pH of the food (De Sena and Jarvis, 1981) and the presence of asperities or a significant microbial load on the product surface providing protection against light (Gómez-López *et al.*, 2005; Elmnasser *et al.*, 2007) may also affect the efficacy of pulsed-light treatment.

The hybridization state of the nucleic acid also appears to affect the susceptibility of viruses to photo-inactivation. Single-stranded nucleic acids are apparently more photosensitive than double-stranded nucleic acids (Sinsheimer *et al.*, 1962; Roberts and Hope, 2003). The superior resistance of double-stranded nucleic acids is likely due to damaged portions of a strand being repaired using the intact opposite strand as a template (Kallenbach *et al.*, 1989). Nucleic acid hybridization state in conjunction with virus size and type appears to have an impact on the effectiveness of inactivation by UV light treatment (Sommer *et al.*, 2001). For example, poliovirus

type 1 (7440 bases in a particle 20–30 nm in diameter) is less sensitive than the MS2 phage (3569 bases in a particle of similar size) to the same treatment (Simonet and Gantzer, 2006). It has also been shown that freezing/thawing before treatment increases the efficiency of the inactivation of adenovirus type 6 by UV light (Nwachuku *et al.*, 2005).

23.2.4 Pulsed light treatments

23.2.4.1 Principle and mode of action

Pulsed light technology has emerged in recent years as a new alternative to thermal treatment for inactivating pathogens and spoilage microorganisms on foods. Its study began during the late 1970s in Japan and a commercial process was patented in 1984 (Hiramoto, 1984). Known by several names in the scientific literature (pulsed-light, pulsed broad spectrum white light, pulsed ultraviolet light, high-intensity broad-spectrum pulsed light, pulsed white light), this technology is based on exposing the food to pulses of light emitted by a xenon lamp. The spectrum is polychromatic (from 200 to 1000 nm) and the pulses are 20,000 times more intense than the sunlight reaching the Earth's surface (Elmnasser *et al.*, 2007).

The FDA has approved pulsed light treatment for the decontamination of food or food-contact surfaces using a xenon lamp emitting wavelengths between 200 and 1000 nm, pulse durations not exceeding 2 ms and cumulative intensity not exceeding 12 J cm^{-2} (FDA, 1996). As is the case for UV light, one advantage of pulsed light is that it does not produce toxic compounds (Gómez-López *et al.*, 2007). Although only a few studies have been carried out on foods, pulsed light does not seem to alter food sensory attributes and nutritional properties (Dunn *et al.*, 1989; Palmieri and Cacace, 2005; Gómez-López *et al.*, 2007; Palgan *et al.*, 2011). Unlike UV light, pulsed light treatment should produce minimal photo-oxidation because of the short pulse duration (Fine and Gervais, 2004), and represents less of a threat to the environment than mercury lamps, since xenon is an inert gas (Gómez-López *et al.*, 2007). Due to its high intensity and short duration, pulsed light

provides effective microbial inactivation at relatively low energy input, penetrating opaque or thick materials better than continuous UV light and causing significantly lower product damage. In addition, it is claimed by the manufacturers of the equipment that pulsed light has a relatively low operating cost (Palmieri and Cacace, 2005). Its main drawback is that sample heating may occur, depending on the treatment (Ozer and Demirci, 2006). Shadowing can also occur, allowing microbes hidden in the food or those deep inside colonies to escape exposure to the light (Hiramoto, 1984). For large-scale applications, the possibility of the emergence of resistant microbes should be investigated.

The specific mechanisms by which pulsed light causes viral inactivation are not understood. However, information is available on bacteria and fungi. The mechanisms involved photochemical and photothermal effects. Indeed, pulsed light inactivates microorganisms partly because the emitted spectrum includes UV light, of which the specific effects are described in Section 23.2.3 (Moraru and Uesugi, 2009). Pulsed light also inactivates microorganisms by increasing the temperature of the treated item, and thus the microorganisms, sufficiently to vaporize a portion of the water from the microorganism. This water then escapes in a steam flow, inducing membrane disruption and causing their death. This overheating has been observed in fungal spores and vegetative cells of bacteria (Wekhof *et al.*, 2001; Elmnasser *et al.*, 2007; Farrell *et al.*, 2009). We postulate that those mechanisms are also involved in virus inactivation by pulsed light.

23.2.4.2 Virus inactivation by pulsed light treatments

Studies of viral inactivation by pulsed light remain rare and difficult to compare because of differences in the equipment used and the type of data reported (Huffman *et al.*, 2000; Lamont *et al.*, 2007; Jean *et al.*, 2011). There is one study of the efficiency of viral inactivation by pulsed light on inert surfaces, but no study published to date of the inactivation of viruses on foods. On stainless steel or polyvinyl chloride coated with foetal

bovine serum to simulate organic matter, titres of HAV and norovirus 1 were reduced by more than 3 $\log_{10}$ cycles (Jean *et al.*, 2011).

23.2.5   Irradiation treatments

23.2.5.1   Principle and mode of action

Food irradiation was conceived in the last years of the nineteenth century, with the discoveries of X-rays by von Roentgen in 1895 and radioactivity by Becquerel in 1896. Also called gamma irradiation, cold pasteurization or irradiation pasteurization (EPA, 2011), food irradiation as a means of destroying bacteria has been studied since 1904 (Josephson, 1983). In 1983, the Codex Alimentarius Commission stated that foods receiving irradiations up to 10 kGy were safe to eat and therefore toxicological testing was no longer necessary. Since then, several countries have approved irradiation for food applications. The US FDA issued a regulation (21 CFR 179.26) that specifies limitations on the fluence permitted for specific applications as well as the type and sources of irradiation used. This regulation allows gamma rays from cobalt-60 or cesium-137, x-rays from x-ray generators and accelerated electron beams from electron beam accelerators. Specific labelling and an international recognized logo (Figure 23.2) must also appear on the package. The statement 'treated with radiation' or 'treated by irradiation' must be displayed (FDA, 2011b).

Irradiation treatments can be grouped into the following three processes based on the reduction of antimicrobial activity obtained: radurization (also called radiation pasteurization, 0.75–2.5 kGy), radicidation (2.5–10 kGy) and radappertization (equivalent to a 12-D heat treatment, 30–40 kGy). Radappertization is not recommended for use in

Figure 23.2   Internationally recognized 'Radura' logo for irradiated foods

foods since it alters their properties significantly (Mendonca, 2002; Ngadi *et al.*, 2012).

X-rays, gamma rays and electron beams are ionizing radiations, meaning they have sufficient energy to knock off electrons from molecules and thereby disrupt both covalent and non-covalent bonds. In the case of viruses, ionizing radiations cause breaks in both RNA and DNA and damage the viral capsid, leading to corruption of the viral genetic code and distortion of viral geometry (Feng *et al.*, 2011). These chemical events may also be a consequence of reactive diffusible free radicals such as hydroxyl radical, hydrogen peroxide or hydrogen, formed by the radiolysis of water (Rahman, 2007b).

23.2.5.2   Virus inactivation by irradiation treatments and factors affecting their effectiveness

Few studies have focused on the effects of irradiation on viruses. In general, a treatment of at least 3 kGy is needed to achieve a 1 $\log_{10}$ reduction of viral titre in foods (Heidelbaugh and Giron, 1969; Mallett *et al.*, 1991; Bidawid *et al.*, 2000a; Jung *et al.*, 2009; Zhou *et al.*, 2011). Murine norovirus and coxsackievirus B-2 seem to be more resistant, since 7 kGy are needed to obtain a 1 $\log_{10}$ reduction (Sullivan *et al.*, 1973; Feng *et al.*, 2011; Sanglay *et al.*, 2011). Doses up to 4.0 kGy and 5.5 kGy are currently approved by the US FDA to control foodborne pathogens in lettuce and molluscs respectively (FDA, 2011b). The use of gamma irradiation is therefore quite impractical at authorized doses as means of inactivating all viruses in fresh products. In addition, no study has been published to date on viral inactivation by irradiation of surfaces.

Since food products can be irradiated in the package, the possibility of post-processing contamination is eliminated. Irradiation is also effective on frozen foods, which increases the threshold dose usable before off-flavour develops. The properties of irradiated foods are similar to those treated with conventional pasteurization technologies. Poultry meat, eggs, red meats, sea products, as well as spices and other frying ingredients are good candidates for decontamination by irradiation. Dissipation of radiation-induced off-flavours or off-odours may occur during storage, and

optimizing packaging conditions and controlling packaging permeability can control changes to sensory properties to a certain extent. Decontamination of foods by ionizing radiation is therefore considered as a safe, efficient, environmentally clean and energy efficient process (Sudarmadji and Urbain, 1972; Luchsinger *et al.*, 1996; Farkas, 1998). Ionization nevertheless requires the establishment of very strict safety rules and is very expensive to implement. Although its safety and effectiveness have been demonstrated, irradiation still has bad press among consumers since it involves nuclear energy. It remains true that irradiation of foods can have a negative impact on proteins, lipids, carbohydrates and vitamins, depending on the dose used. Indeed, irradiation of proteins (containing phenylalanine, tyrosine and methionine) and unsaturated lipids leads to the development of off-flavours, in addition to decreasing nutritional value. Excluding oxygen and applying antioxidants decreases lipid oxidation. Irradiation can also de-polymerize high-molecular-weight carbohydrates into smaller units, resulting in softening of fruits and vegetables. The effect of irradiation on vitamins depends on the fluence used. Thiamine is considered one of the most labile to irradiation (Farkas, 1998; Rahman, 2007b).

The inactivation of foodborne microorganisms by ionizing radiation depends on the irradiation fluence, the type of virus, the temperature and the food composition (Mendonca, 2002). The degree of inactivation obtained is generally proportional to fluence, although an inverse relationship exists between the fluence required and viral genome size (Feng *et al.*, 2011). Low temperatures tend to protect viruses against irradiation (Jung *et al.*, 2009). Finally, the effectiveness of gamma irradiation has been found greatly affected by the presence of proteins, which apparently capture free OH radicals before they can damage viral nucleic acid and capsids (de Roda Husman *et al.*, 2004).

23.2.6 High-pressure treatments

23.2.6.1 Principle and mode of action

The first studies of the effects of high pressure on foods are those of Hite, who reported before the beginning of the twentieth century that milk 'kept sweet for longer' after a pressure treatment of 600 MPa for 1 hour at room temperature (Hite, 1899). The first pressure-treated foods became available commercially in Japan in the early 1990s (Patterson, 2005). Today, high-pressure processing is also known as high hydrostatic pressure or ultra-high-pressure processing. This treatment consists of subjecting liquid or solid foods, with or without packaging, to pressures between 50 and 1000 MPa (0.5–10 kbar or 7–145 kpsi), to inactivate microbial cells. The temperature variation is minimal (3 °C per 100 MPa, depending on the food composition) and all parts of the product are subjected to the same pressure at exactly the same time, unlike heat processing in which temperature gradients are created. High-pressure systems and their effects have been reviewed (Hogan *et al.*, 2005). Although this technology allows significant extension of food shelf life, it does not sterilize and foods must therefore be refrigerated in order to maintain their sensory characteristics and microbiological stability (Hogan *et al.*, 2005; Patterson, 2005; Rendueles *et al.*, 2011).

The use of high pressure for food has not been subject to regulation by the US FDA. In Europe, high-pressure treated foodstuffs may be considered 'novel foods' in accordance with EC regulation 258/97. Several products have been evaluated and approved for commercialization on a national scale, such as high-pressure pasteurized orange juice (France), cooked ham (Spain), oysters (Great Britain) and fruits (Germany) (Eisenbrand, 2005). High pressure could be used to inactivate bacteriophages in milk used for cheese making (Müller-Merbach *et al.*, 2005).

The principal advantage of high pressure is that foods can be processed at room temperature. This ensures minimal change in the nutritional quality and organoleptic properties of the processed food. Both packaged and unpackaged foods may be treated. In addition, high pressure has been proven to reduce the total energy requirement by at least 20% compared to conventional sterilization by heat (Ngadi *et al.*, 2012). Nevertheless, some adverse effects associated with high-pressure treatment have been reported. Colour changes

may occur in some foods. For example, fresh meat may present a cooked appearance, due to changes in myoglobin structure (Hogan *et al.*, 2005). A major drawback of high pressure is the high cost of the equipment. High pressure is still considered an expensive specialized technology, since a commercial vessel may cost GBP 500,000 to 1 million, depending on the size (Patterson, 2005).

In terms of mechanism of action, high pressure appears to inactivate viruses by disrupting the capsid structure. At pressures of 200 MPa and higher, ionic and hydrophobic bonds responsible for maintaining the tertiary and quaternary structure of capsid proteins may be disrupted without affecting hydrogen bonds or covalent bonds (Balny and Masson, 1993). Disruption of viral capsid structure likely impairs capsid functions such as attachment, receptor binding and entry of the virus into the host cell. High pressure does not seem to damage the genetic material, of which the integrity depends on covalent and hydrogen bonds (Tang *et al.*, 2010; Lou *et al.*, 2011a, 2011b). One consequence of this is that quantitative detection of the viral genome by PCR does not measure the inactivation effect of high pressure (Diez-Valcarce *et al.*, 2011).

23.2.6.2 Virus inactivation by high-pressure treatments and factors affecting their effectiveness

The resistance to high pressure, or baro-resistance, of viruses is highly variable. In general, a treatment of 500 MPa for 1 min should be sufficient to obtain at least a 3 $\log_{10}$ reduction of active virus in or on foods (Calci *et al.*, 2005; Kingsley *et al.*, 2007; Black *et al.*, 2010; Kovač *et al.*, 2012). Nevertheless, bacteriophages φX174, MS2, T4 and c2 resist pressures up to 500 MPa (Smiddy *et al.*, 2006; Sharma *et al.*, 2008).

The effectiveness of high pressure for inactivating noroviruses has been investigated using *in vivo* assays. In a study by Gogal *et al.* (2011), a treatment of 400 MPa for 5 min rendered this virus incapable of infecting mice. Similarly, oysters spiked with 10^4 infectious units of Norwalk virus (GI.1) and subsequently subjected treated at

600 MPa for 5 min at 6 °C did not cause illness in human volunteers, while treatment at 400 MPa was apparently not destructive to this virus (Leon *et al.*, 2011). In study by Lou *et al.* (2012), virus-like particles of human norovirus GII.4 easily resisted pressures of up to 600 MPa. The capsid of the GII.4 strain might be more resistant to high pressure than that of the GI.1 strain (Lou *et al.*, 2012), just as coxsackievirus B-5 is more resistant than coxsackievirus A-9.

The destructiveness of high pressure to viruses in foods depends on several parameters including pressure, temperature and composition of the food matrix. Inactivation is generally proportional to pressure, and pressure has a greater impact than treatment time (Arcangeli *et al.*, 2012). Fat, protein, carbohydrate, salinity and acidity have all been shown to provide protection against the destructive effects of high pressure (Chen *et al.*, 2004; Murchie *et al.*, 2007; Kingsley and Chen, 2008; Lin *et al.*, 2010). Finally, resistance to high pressure varies within related taxonomic groups or even strains (Baert *et al.*, 2009a).

23.2.7 Other physical treatments

23.2.7.1 Ultrasound

Ultrasound is a cyclic sound pressure wave with a frequency above the human audibility range (20 kHz or more). It is also called sonication or ultra-sonication. It is widely used to disrupt particles including living cells. Despite the low number of reported studies, it appears safe to say that this treatment is insufficient to inactivate significant numbers of viruses (Scherba *et al.*, 1991; Su *et al.*, 2010; Fraisse *et al.*, 2011).

23.2.7.2 Pulsed electric field treatment

Treatment by pulsed electric fields involves the application of high-voltage electric fields in very short pulses to food products held between a set of electrodes. This technology is best applied to liquid and semi-liquid foods such as juices, milk, yogurt, soup or liquid eggs, all of which contain ions that are electric charge carriers and hence conduct electrical current. This processing can vary

in terms of electric field strength, pulse waveform, temperature, pressure and time of exposure. Although the antibacterial effects of pulsed electric field treatment are well documented, little information is available on its antiviral effects. The few studies carried out on enteric viruses show that pulsed electric fields of strengths up to $29\,kV\,cm^{-1}$ do not produce the minimal 3 $\log_{10}$ cycle reduction of infectious particles required to warrant further attention (Ingram and Page, 1953; Khadre and Yousef, 2002; Drees *et al.*, 2003). More in-depth studies are still needed to evaluate the potential of this promising technology as a new method of cold processing for the inactivation of enteric virus in foods.

23.2.7.3 Modified atmosphere packaging
Modified atmosphere packaging can inhibit spoilage caused by bacteria and fungi, thereby extending food shelf life. However, it cannot inactivate enteric viruses (Bidawid *et al.*, 2001).

23.2.7.4 Reduced water activity
Reducing the water activity of a food slows down the growth rate of bacteria and other microorganisms. However, water activity does not appear to have much effect on viruses except for poliovirus, which seems to be less resistant than other enteric viruses (Abad *et al.*, 1994; Baert *et al.*, 2009a). Studies conducted on foods corroborate this observation (Konowalchuk and Speirs, 1975; Stine *et al.*, 2005).

23.3 Chemical treatments

In general, the factors to consider when choosing a chemical disinfectant include the organic load in the process water, the type of organic material present, exposure time, concentration, temperature, water pH and chemistry, type of microbial organisms present and product (e.g. vegetable) topography (Hirneisen *et al.*, 2010). This section presents the conventional hypochlorous acid treatment as well as alternative chemicals such as chlorine dioxide, ozone and peracetic acid.

23.3.1 Washing
Simply washing vegetables reduces virus numbers typically by about 1 $\log_{10}$ regardless of the washing method used (Bae *et al.*, 2011; Fraisse *et al.*, 2011). Despite the limited reduction, washing is a basic common-sense step to apply unconditionally in order to reduce the numbers of virions in food preparations and in viral transmission routes. The effectiveness of this step can be enhanced by adding trisodium phosphate to the water (Su and D'Souza, 2011).

23.3.2 Hypochlorous acid
Halogens are molecules of non-metal elements (group 17 of the periodic table) including most notably chlorine (Cl). The halogens are strong oxidizers, meaning they tend to gain electrons and form anions such as Cl^-, which makes them suitable as sanitizing and disinfecting agents (Slowinski *et al.*, 2011). Chlorine compounds are commercially available as hypochlorites (mainly sodium and calcium salts of hypochlorous acid HClO, i.e. NaClO and $Ca(ClO)_2$), chlorine gas Cl_2, chlorine dioxide ClO_2 and chloramines. Chloramines are slow-release forms derived from ammonia by substitution of one, two or three hydrogen atoms with chlorine atoms.

23.3.2.1 Principle and mode of action
According to the US FDA regulation, the use of sodium or calcium hypochlorite is allowed up to $200\,mg\,L^{-1}$ (equivalent to 200 ppm of available chlorine) for food-contact surface decontamination (FDA, 2011e) and for food decontamination (FDA, 2010). It is applied to fresh produce generally at a temperature of 12–21 °C, pH 5–7 for 2–10 s as a spray or for 30 s to 5 min as flume water (Hirneisen *et al.*, 2010).

Hypochlorous acid is the active form of chlorine in water. Its standard potential of oxidation is +1.49 V (Ibanez *et al.*, 2007). When dissolved in water, sodium hypochlorite (Equation 23.1), calcium hypochlorite (Equation 23.2) and chlorine

gas (Equation 23.3) all generate hypochlorous acid. Sodium and calcium hypochlorites are chlorine in alkaline form. Sodium hypochlorite is usually sold as a liquid whereas calcium hypochlorite is usually sold as a powder, granules or tablets (EPA, 1983).

$$NaOCl + H_2O \rightarrow HOCl + Na^+ + OH^- \qquad (23.1)$$

$$Cl_2 + H_2O \rightarrow HOCl + HCl \qquad (23.2)$$

$$Ca(OCl)_2 + 2H_2O \rightarrow 2HOCl + Ca^{2+} + 2OH^- \qquad (23.3)$$
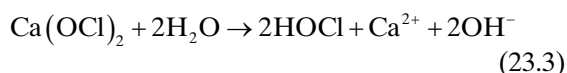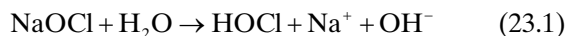
Once HOCl and OH$^-$ are formed, an additional reaction occurs (Equation 23.4) generating a hypochlorite ion (OCl$^-$). The equilibrium of this chemical reaction is pH dependent and both compounds coexist in equal proportions at pH 7.4 (Holah, 2003; AWWA, 2006).

$$HOCl + OH^- \leftrightarrow OCl^- + H_2O \qquad (23.4)$$

Although hypochlorous acid is effective in many situations, it has several disadvantages. It may react with natural organic matter to produce a variety of toxic byproducts including trihalomethanes such as chloroform, considered as potential carcinogens (Sedlak and von Gunten, 2011). Other classes of byproducts also may be produced such as haloacetic acids, haloacetonitriles or cyanogen halides (Singer, 1994). Peptides in water are of greater concern than free amino acids because chlorinated peptides are more stable than chlorinated derivatives of amino acids (Sharma and Sohn, 2012).

In terms of mechanism of action, chlorine seems to act differently depending on the virus type (RNA or DNA). On RNA viruses, chlorine seems to act on the genetic material, even at concentrations below 1 mg L^{-1} (O'Brien and Newman, 1979), causing the loss of the 5′ non-translated region (Li *et al.*, 2002). This may explain why few authors have ever detected inactivated poliovirus with intact nucleic acid by PCR (Blackmer *et al.*, 2000). While the sequence chosen for amplification was intact, some regions were probably lost. Chlorine may also act by altering the capsid proteins at doses above 1 mg L^{-1} (Li *et al.*, 2002). In the case of DNA viruses, inactivation is due to damage to capsid proteins, especially those that direct replication cycle processes after attachment to the host cell (Page *et al.*, 2010), possibly causing the release of the viral genome into the surrounding medium. It is not known whether these differences are due to different binding mechanisms of the viruses, different capsid protein compositions, or reactions associated with the higher chlorine exposures required to inactivate single-stranded RNA viruses (Page *et al.*, 2010).

23.3.2.2   Virus inactivation by hypochlorous acid and parameters affecting its efficiency

Published studies show clearly that 200 mg L^{-1}, the highest chlorine concentration allowed, is not effective (reduces viral infectious titre by less than 3 log$_{10}$) in removing viral contaminants from fresh produce (Dawson *et al.*, 2005; Casteel *et al.*, 2008; Baert *et al.*, 2009b; Fraisse *et al.*, 2011). Similar results have been obtained for surface decontamination (Eterpi *et al.*, 2009; Kim *et al.*, 2012). Baert and colleagues emphasize that chlorine treatment of fresh produce is more useful for preventing cross-contamination during the washing process than for reducing the number of infectious units present on fresh produce (Baert *et al.*, 2009b). Since porcine parvovirus is one of the most resistant viruses to chlorine treatment, it could be used as an indicator of chlorine treatment efficacy.

The destructive effect of halogens on viruses is affected mostly by pH, temperature and the presence of organic matter. Since the effect depends on hypochlorous acid concentration, efficacy is greater at acidic pH. Higher temperature improves efficacy (Kelly and Sanderson, 1958; Jean *et al.*, 2003; Lukasik *et al.*, 2003; Kahler *et al.*, 2010), while the presence of organic matter in the viral sample tends to decrease efficacy (Park and Sobsey, 2011). When conditions are not optimal, it is possible to compensate for low efficacy by increasing the concentration of the active agent.

23.3.3 Chlorine dioxide

23.3.3.1 Principle and mode of action

Chlorine dioxide is the most widely accepted English name for the molecule ClO_2. The terms chlorine oxide, anthium dioxide, chlorine (IV) oxide, chlorine peroxide, chloroperoxyl and chloryl radical are also used (Knapp and Battisti, 2000). The compound may be used at residual concentrations not exceeding $3\,mg\,L^{-1}$ as an antimicrobial agent in water for poultry processing or to wash fruits and vegetables that are not raw agricultural commodities. The treatment of fruits and vegetables with chlorine dioxide must be followed by a potable water rinse or by blanching, cooking or canning (FDA, 2011a).

Several methods of generating chlorine dioxide are permitted by the FDA (2011a), in particular passing chlorine gas through an aqueous solution of sodium chlorite using a mechanical generator:

$$2NaClO_2 + Cl_2 \rightarrow 2ClO_2 + 2NaCl \qquad (23.5)$$

Chlorine dioxide at room temperature is an explosive yellowish-green gas, existing primarily as a free radical. One of its most important physical properties is its high solubility in water, up to $20\,g\,L^{-1}$ or approximately 10 times more soluble than chlorine (EPA, 1999; Sharma and Sohn, 2012).

Chlorine dioxide disinfects by oxidation (standard potential of $+1.28\,V$) but it does not chlorinate (EPA, 1999; Ibanez *et al.*, 2007). Its main advantage over other chlorine-based disinfectants is that it reacts primarily with tertiary amines, phenols and aromatic amines and limits the formation of harmful trihalomethanes and haloacetic acids when reacting with natural organic matter. However, since it is explosive under pressure, it has to be produced on-site (Sharma and Sohn, 2012).

Chlorine dioxide supposedly reacts with the 5' un-translated region of the RNA genome (Jin *et al.*, 2012), thus impairing the ability of the molecule to act as a template for RNA synthesis (Alvarez and O'Brien, 1982). At concentrations above $1\,mg\,L^{-1}$, it may affect capsid proteins without promoting virolysis (Li *et al.*, 2004; Nowak *et al.*, 2011).

23.3.3.2 Virus inactivation by chlorine dioxide and parameters affecting its efficiency

Based on studies in buffer, a concentration of $3\,mg\,L^{-1}$ of chlorine dioxide is sufficient to inactivate adenovirus, feline calicivirus, HAV and coxsackievirus B-5 (Thurston-Enriquez *et al.*, 2005; Zoni *et al.*, 2007). However, no study has been conducted on foods to date. Studies of effectiveness on surfaces suggest that chlorine dioxide should be used at concentrations reaching $500\,mg\,L^{-1}$ for 5 minutes to ensure sufficient destruction of viruses (Sabbah *et al.*, 2010). Additional studies are needed to validate these results.

Effectiveness may be affected by different factors including pH, temperature and suspended organic matter. Inactivation is enhanced with increasing alkalinity and with increasing temperature. Suspended matter and pathogen aggregation reduce its disinfection efficiency (EPA, 1999; Thurston-Enriquez *et al.*, 2005).

23.3.4 Ozone

23.3.4.1 Principle and mode of action

Interest in ozone (O_3) has expanded in recent years in response to consumer demand for 'greener' food additives, regulatory approval hurdles and increasing acceptance of ozone as an environmentally friendly technology (O'Donnell *et al.*, 2012). The use of ozone in the food industry has been reviewed (Khadre *et al.*, 2001; Guzel-Seydim *et al.*, 2004). Ozone (CAS RN 10028-15-6) may be used safely as an antimicrobial agent in the treatment, storage and processing of foods, including meat and poultry, in the gaseous or aqueous phase (FDA, 2011c). It has been suggested recently that ozone be used for other applications in the food sector such as disinfection of food surfaces and sanitation of food plant equipment (Guzel-Seydim *et al.*, 2004).

Ozone is produced commercially by passing electrical discharges or ionizing radiation through air or oxygen. Pure ozone is an unstable pale blue gas with a characteristic pungent odour. Although it has an oxidation reduction potential of $+2.07\,V$, which is much higher than those of chlorine, hypochlorous acid or hydrogen peroxide, the

reactivity of ozone is due mainly to the great oxidizing power of its free radicals, namely hydroperoxyl ($\cdot HO_2$), hydroxyl ($\cdot OH$) and superoxide ($\cdot O_2^-$), which are formed spontaneously by the decomposition of ozone in water at alkaline pH (Hoigné and Bader, 1975; Khadre et al., 2001).

High reactivity and the absence of harmful residues (excess ozone self-decomposes rapidly to oxygen) make ozone a very attractive agent for ensuring the quality and microbiological safety of foods (Kim et al., 1999). Ozone is produced on-site, eliminating the cost of storage and transportation (Khadre et al., 2001). Unfortunately, ozone is a toxic gas that can cause severe illness and even death if inhaled in large quantities (Guzel-Seydim et al., 2004). Moreover, ozone reacts with lipids and proteins (Khadre et al., 2001; Ölmez, 2012). It is so reactive that it would probably be more effective for viral inactivation on pre-cut/pre-shredded produces because the surface area is increased and the area for ozone to interact on produce is therefore increased (Hirneisen et al., 2011).

As an antiviral disinfectant, the primary mechanism of action of ozone appears to be structural damage to the viral capsid, which subsequently loses its virus-host-cell specificity and therefore its infectivity. Nucleic acids seem to be a secondary site of attack (de Mik and de Groot, 1977; Kim et al., 1980; Sproul et al., 1982; Murray et al., 2008).

23.3.4.2   Virus inactivation by ozone and parameters affecting its efficiency

The few studies on viral inactivation show that treatment for 1 min is insufficient to achieve a 3 $\log_{10}$ reduction or better by washing with water containing ozone at a concentration of $6.25\,mg\,L^{-1}$ (Hirneisen et al., 2011). The decomposition of ozone in the aqueous phase of foods is so rapid that its antiviral action may be presumed to take place mainly at the surface (Hoigné and Bader, 1975). The potential of gaseous ozone as an antiviral disinfectant has been evaluated. It appears highly effective for the inactivation of airborne viruses (Tseng and Li, 2006; Hudson et al., 2007).

In general, the antiviral activity of ozone may be affected by three factors, namely temperature, pH and ozone-consuming compounds. Effectiveness

seems to decrease as temperature increases (Roy et al., 1982; Herbold et al., 1989), although no difference was observed between 5 and 20 °C in at least one study (Lim et al., 2010). Viruses seem more resistant to inactivation by ozone at low pH than at neutral pH (Roy et al., 1982), although Lim and colleagues noted that MNV-1 was more rapidly inactivated at pH 5.6 than at pH 7. Finally, the composition of the food matrix can interfere with inactivation by providing an abundance of reducing compounds with which ozone reacts preferentially (Hirneisen et al., 2011).

23.3.5   Peroxyacids

23.3.5.1   Principle and mode of action

Peroxyacetic acid or peracetic acid is the most common peroxyacid sold for sanitization. The US FDA has approved its use at concentrations up to $200\,mg\,L^{-1}$ for sanitizing certain food products (especially fruits and vegetables) and food-contact surfaces, and up to $80\,mg\,L^{-1}$ in washing water (FDA, 2010, 2011d, 2011e).

Peracetic acid is produced from the reaction of acetic acid (CH_3CO_2H) with hydrogen peroxide (H_2O_2) in the presence of sulphuric acid (H_2SO_4), which acts as a catalyst. This reaction is not complete and reaches equilibrium. Peracetic acid is thus available as a mixture containing acetic acid (CH_3CO_2H), hydrogen peroxide (H_2O_2), peracetic acid (CH_3CO_3H) and water (H_2O) (Equation 23.7; González-Aguilar et al., 2012). It is a clear, colourless liquid with a strong pungent acetic acid odour.

$$CH_3CO_2H + H_2O_2 \leftrightarrow CH_3CO_3H + H_2O \quad (23.7)$$

In this chapter, peracetic acid solution refers to the mixture of the four components described above. The main advantage of peracetic acid and the other peroxyacids is that they do not release toxic compounds into the environment. Moreover, the efficacy of peracetic acid appears indifferent to the presence of organic matter (Kitis, 2004). However, there is a lack of data on the inactivation of viruses on foods and food-contact surfaces.

Very few studies have been published on the mechanism of action of peracetic acid on viruses.

Its disinfectant activity is based on the release of active oxygen (González-Aguilar *et al.*, 2012), which mainly alters capsid structure (Maillard *et al.*, 1995, 1996b; Wutzler and Sauerbrei, 2000), leading to damage to the genome (Maillard *et al.*, 1996a).

23.3.5.2 Virus inactivation by peracetic acid and parameters affecting its efficiency

Few studies have been conducted on the efficacy of peracetic acid against viruses. It is presumed that the activity of this mixture is due mainly to peracetic acid, since hydrogen peroxide must be present at concentrations of at least 10,000 mg L^{-1} in order to inactivate viruses (Mbithi *et al.*, 1990; Hall and Sobsey, 1993; Best *et al.*, 1994; Koivunen and Heinonen-Tanski, 2005; Eterpi *et al.*, 2009; Pottage *et al.*, 2010; Li *et al.*, 2011).

Reductions of infectious titres of HAV, feline calicivirus and murine norovirus reached 1–3 log$_{10}$ cycles on lettuce (the only food tested) treated with peracetic acid at concentrations of 100–250 mg L^{-1} for a few minutes (Baert *et al.*, 2009b; Fraisse *et al.*, 2011). This compound would therefore be more useful for preventing cross-contamination during the washing process rather than reducing the number of infectious particles present on foods. Moreover, studies conducted on surfaces used concentrations about 10 times higher than those permitted by the FDA (Mbithi *et al.*, 1990; Eterpi *et al.*, 2009; Magulski *et al.*, 2009; Sabbah *et al.*, 2010). At these concentrations, peracetic acid is effective against most viruses except for HAV, which could be used as a reference for disinfection by peracetic acid. The presence of organic material has little impact on the antiviral activity (Wutzler and Sauerbrei, 2000; Poschetto *et al.*, 2007; Baert *et al.*, 2009b). More studies are needed to specify the nature of the activity of peracetic acid under different conditions.

23.3.6 Other chemical agents

23.3.6.1 Iodophores

Iodophores are solutions composed of iodine and non-ionic surfactants or carriers. The US FDA approves their use for sanitizing food-handling equipment (FDA, 2011e). In addition, even though they are not approved for direct contact with foods, they might have some usefulness for treatment of produce items that are peeled before consumption (Ayala-Zavala and Gonzales-Aguilar, 2009). Today, iodophores are applied mainly as topical antiseptics for medical and veterinary purposes. Their use has been reviewed (Springthorpe and Sattar, 1990).

23.3.6.2 Surfactants

Surfactants are usually organic compounds that are amphiphilic, meaning they contain a hydrophobic portion (called the tail) and a hydrophilic portion (called the head). These molecules can be classified according to the polar head group: non-ionic surfactants such as Nonidet P-40, Triton X-100 or polysorbates (Tween 20) have no charged groups, anionic surfactants such as sodium dodecyl sulphate (SDS) have a negatively charged group and cationic surfactants such as quaternary ammonium compounds have a positively charged group.

Non-ionic and anionic surfactants have recently been reported as enhancers of antiviral sanitization in fresh produce. The combination of these molecules with a commonly used sanitizer enhanced the efficiency of the removal of viruses from fresh produce by approximately 100-fold. The surfactants used were SDS, Nonidet P-40, Triton X-100 and Tween 20 (Predmore and Li, 2011). Since SDS is an FDA-approved food additive and polysorbates are considered 'generally recognized as safe' (GRAS) by the FDA, the implementation of this novel sanitization strategy would be a feasible approach to reduce viral load in fresh produce.

Quaternary ammonium compounds are a large group of cationic surfactants in which the hydrogen atoms in the ammonium group are replaced by alkyl and/or aryl groups. Typically, at least one of the alkyl groups is a long hydrophobic carbon chain, which increases its surface-active properties. One of the most widely used quaternary ammonium compounds is benzalkonium chloride (alkyldimethylbenzylammonium chloride). The most widespread use of these compounds is as disinfectants of hard

surfaces (Springthorpe and Sattar, 1990). Their effect on enteric viruses is uncertain.

Many researchers report no destructive effect (Doultree *et al.*, 1999; Rutala *et al.*, 2000; Belliot *et al.*, 2008; Eterpi *et al.*, 2009; Whitehead and McCue, 2010; Tuladhar *et al.*, 2012), while others have observed 2–3 $\log_{10}$ reductions of infectious titre after treatment at concentrations of 0.5 mg mL^{-1} (Su and D'Souza, 2012).

23.3.6.3   Alcohols

Alcohol-based sanitizers are generally effective for reducing the numbers of enteric viruses by at least 3 $\log_{10}$ cycles on human hands (Gehrke *et al.*, 2004; Kampf *et al.*, 2005, 2011; Macinga *et al.*, 2008). However, the use of such sanitizers does not replace hand washing with soap and water, although it might complement it. Hospitals have already associated the use of alcohol-based hand sanitizer alone without hand washing with an increase in the number of outbreaks of gastro-enteritis (Blaney *et al.*, 2011). The true efficacy of alcohol as an antiviral disinfectant on surfaces remains uncertain (Magulski *et al.*, 2009; D'Souza and Su, 2010).

23.3.6.4   Acids

Although acids are well known as antimicrobial substances, they do not efficiently inactivate enteric viruses. Tolerance to acid is an adaptive feature of foodborne viruses, since they must withstand acidic pH during passage through the stomach. Nevertheless, the combination of an organic acid (levulinic acid) and SDS reduces infectious viral titre by more than 3 $\log_{10}$ on stainless steel surfaces after 5 minutes of exposure. In addition, the presence of organic matter did not significantly affect sanitizer efficacy (Cannon *et al.*, 2012). This subject has been reviewed by Baert *et al.* (2009a).

23.4   Conclusions and future outlook

Heat processing is an effective means of inactivating viruses in foods. It is not applicable to all products however; in view of the increasing popularity of minimally processed foods, other agents must be examined. Chlorine-based disinfectants are widely used despite their tendency to release toxic byproducts, since their antiviral activity on foods and food-contact surfaces is indisputable. In view of the increasing numbers of outbreaks of gastroenteritis associated with changing food consumption habits, it is obvious that novel processes such as cold pasteurization are needed in order to target viruses while maintaining the nutritional and organoleptic value of foods. The new processes also must be less threatening to the environment. High-risk foods are becoming an ongoing challenge to food processors, which have yet to come up with an acceptable way of completely inactivating enteric viruses in foods or on food-contact surfaces. Further research on the effects of viral inactivation techniques and agents in various food matrices is urgent. Finally, it should not be forgotten that food-processing technology must never be used as a substitute for good agricultural practices, good manufacturing practices and proper sanitation.

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24

Use of Synbiotics (Probiotics and Prebiotics) to Improve the Safety of Foods

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Summary

In recent years, the number of functional dairy products enriched with live probiotic microorganisms has increased exponentially since it is known that these can confer health benefits to the host. One of the most important mechanisms by which probiotics can act in the prevention of disease is through their capacity to produce antimicrobial substances such as lactic acid and bacteriocins. These can prevent the growth of pathogenic microorganisms in foods, meaning that probiotics could therefore be used as a tool to prevent foodborne diseases. Currently, some foods are supplemented with prebiotics (non-digestible oligosaccharides) in order to favor the growth of selected probiotics in the gastrointestinal tract. Here, we will discuss the current and future uses of synbiotics (probiotics and prebiotics) in order to increase the safety of food.

24.1   Introduction

Lactic acid bacteria (LAB) constitute a phylogenetically heterogeneous group of ubiquitous microorganisms that are naturally present in high-nutrient-containing organic products such as foods and occupy a wide range of ecological niches ranging from the surface of plants to the gastro-urogenital tract of animals. Currently, the LAB group includes a large number of cocci and bacilli, such as species of the genera *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella*, that normally contain a G + C content inferior to 55% in their chromosomal DNA. Although quite diverse, the members of this group have various characteristics in common, including: (1) Gram-positive; (2) facultative anaerobes; (3) non-sporulating; (4) non-motile; and (5) possess the capacity to convert sugars into lactic acid (Nouaille *et al.*, 2003). LAB are one of the most important industrial groups of bacteria that are widely used in food production, health improvement, and production of macromolecules, enzymes, and metabolites.

From a historical point of view, LAB have been used since ancient times in food fermentation processes and preservation. Since the 1980s, many efforts have been made to better understand the molecular basis of LAB's technological properties in order to control the industrial processes involving these important microorganisms. Due to their lack of pathogenicity, most LAB species have received the GRAS (generally recognized as safe) status by the US Food and Drug Administration. In addition to their important technological properties in food production (production of lactic acid, decrease of lactose, improvement of organoleptic, and physical characteristics), various species of LAB such as *Lactobacillus casei*, *Lactobacillus delbrueckii*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, and *Lactobacillus reuteri*, have been shown to possess therapeutic properties. Using mostly animal models, they have demonstrated their ability to prevent the development of some diseases and to promote beneficial effects in human and animal health (LeBlanc *et al.*, 2008).

Because of their documented beneficial effects, certain strains of LAB have been designated as *probiotic* that have been defined by the FAO/WHO as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' (FAO/WHO, 2001). Some of the health benefits which have been claimed for probiotics include: improvement of the normal microbiota, prevention of infectious diseases and food allergies, reduction of serum cholesterol, anticarcinogenic activity, stabilization of the gut mucosal barrier, immune adjuvant properties, alleviation of intestinal bowel disease symptoms, and improvement of the digestion of lactose in intolerant hosts (del Carmen *et al.*, 2012). The most common strains used as probiotics are members of lactobacilli, enterococci, and bifidobacteria groups (Ouwehand *et al.*, 2002).

Currently, many products containing probiotics are available on retail shelves throughout the world because of the increase in consumer demand for healthier natural foods that can increase their overall well-being. The specific health effects of selected probiotic strains have been confirmed by well-documented double-blind controlled human clinical trials and are becoming increasingly accepted. However, many proposed beneficial effects of probiotics still need further research and more information about their mechanisms of action is required in order to confirm that they can be useful in the prevention and treatment of other specific diseases.

During the last years, research on LAB has been dominated by advances in molecular biology as well as in genetic engineering and genomic technologies. These advances have increased the understanding of their taxonomy, their ecological role, the extent of horizontal gene transfer among them, their metabolic potential, and the interactions with other microorganisms in their environment and with host cells. Furthermore, progresses in recombinant DNA techniques now enables the genetic engineering of previously difficult species allowing the development of

genetically modified LAB for novel applications such as beneficial compound release, vaccines, or even the development of designed probiotics (LeBlanc *et al.*, 2012).

24.2 Probiotics

A significant part of the world population suffers gastrointestinal diseases caused by pathogenic bacteria; however, numerous studies regarding the beneficial effects of probiotics are currently in progress (Donkor *et al.*, 2007). One of the beneficial effects attributed to probiotics is the inhibition or prevention of certain pathogen infections, many of which may arise from food. According to Dave & Shah (1997), to produce the desired benefits probiotics should be present in the product in viable counts during their whole shelf-life. The minimum dose able to assure beneficial effects should therefore be in the range 7–9 log CFU mL^{-1}. The viability of probiotics in commercial preparations is also affected by several factors such as temperature, acidity, and the presence of other microorganisms and oxygen (Shah, 2000).

Potential mechanisms of action by which probiotic microorganisms might exert their protective or therapeutic effect against enteric pathogens include modulation of the mucosal and systemic immune system and displacement of potential pathogens via non-immune mechanisms such as the stabilization of the gut mucosal barrier, increasing the secretion of mucus, interfering with their ability to colonize and infect the mucosa, competing for nutrients, influencing the composition and activity of the gut microbiota, and the production and secretion of specific low-molecular-weight antimicrobial substances (bacteriocins) and other agents (Delgado *et al.*, 2007).

During the last two decades, several microorganisms (including bacteria and yeast) have been tested for their probiotic potentials. A recent study evaluating different bacteria showed that lactobacilli were more efficient in inhibiting pathogens than bifidobacteria and strains from other genera (Chapman *et al.*, 2012). These authors also suggested that using a probiotic mixture might be more effective than a single strain against gastrointestinal pathogens, and that creating a mixture using species and strains with different effects against different pathogens may have a greater spectrum of action.

From a technological point of view, interactions between probiotic microorganisms are very important for the correct development of flavor and texture of functional fermented dairy products (Martin *et al.*, 2011; Oliveira *et al.*, 2012a), being responsible for variations in the amounts of organic acids, volatile compounds, and exopolysaccharides released during manufacture.

Considering the effect of probiotics as immune adjuvants, they can modulate the inflammatory response, stimulate the production of certain cytokines and the activity of cells such as macrophages and neutrophils, regulate NK cell activity, and enhance specific antibody responses, especially mucosal secretory IgA (de Moreno de LeBlanc and Perdigon, 2010; Castillo *et al.*, 2011).

A recent study using a mouse model of *Salmonella enterica* serovar Thyphimurium infection showed that there is no specific or unique mechanism by which a probiotic bacterium can exert their protective or therapeutic effect (de Moreno de LeBlanc *et al.*, 2010a). The continuous administration of *L. casei* CRL431 to mice (before and after infection) diminished the numbers of *Salmonella* in the intestine as well as preventing its appearance in the liver. The probiotic administration also lowered neutrophil infiltration (and thus decreased intestinal inflammation), activated the macrophage phagocytic activity, and increased the number of Immunoglobulin-A (IgA) producing cells in the lamina propria of the small intestine causing the release of anti-*Salmonella* s-IgA in the intestinal fluids.

The modulation of the immune response by probiotic administration was also evaluated in the same mouse model of *Salmonella*. Probiotic administration before the infection improved the production and secretion of certain cytokines in the Peyer's patches, the inductor site of the gut immune response. It was also demonstrated that it is important to give the probiotics on

a continuous basis after the infection to regulate the inflammatory response, mainly in the immune effector site of the gut (lamina propria) (Castillo *et al.*, 2011). These changes in the cytokine profiles were also associated with variations in toll-like receptor (TLR) expressions at the intestinal level.

In other work that was performed using a mouse model of *Salmonella* infection, mice received a probiotic fermented milk (PFM) containing *L. casei* DN-114 001 and showed a decrease of the severity of the infection (de Moreno de LeBlanc *et al.*, 2010b). These observations were accompanied by increased number of IgA producer cells in the lamina propria of the small intestine and total IgA in the intestinal fluid of mice fed with PFM. The analysis of TLRs in this model showed that the number of TLR-4+ cells decreased in mice given probiotic fermented milk continuously or post-infection, which could be related to the decrease in the severity of the infection for these groups where the pathogenic bacteria growth in spleen and liver decreased faster than in the infection control. It is known that TLR-4 recognize the lipopolysaccharide, the principal component of the membrane of Gram-negative bacteria. The activation of this receptor initiates an innate immune response leading to the induction of pro-inflammatory mediators, but then leads to the suppression of its own mRNA expression during *Salmonella* infection (Tötemeyer *et al.*, 2003). In the same previous work, it was reported that newborn mice that received the same probiotic fermented milk after weaning showed a beneficial effect against *S.* Typhimurium (de Moreno de LeBlanc *et al.*, 2010b).

Considering that many of the intestinal infections in humans came from infected food, some beneficial properties attributed to probiotic supplementation besides resistance to infectious disease are applied to farm animal. The use of probiotics is a practice that is not only used to avoid infection as a replacement therapy for antibiotics, but also improves the consumption of dairy products (Lehloenya *et al.*, 2008; Gaggia *et al.*, 2010). There are many studies using probiotics in animals where different mechanisms were attributed to their beneficial effects.

Different genera and species of bacteria and some yeasts are also used as probiotics in animal studies. Most experiments were performed with piglets and the reduction of diarrhea by probiotics; this is the main problem experienced by piglets during the first weeks after weaning, with great importance for production (Taras *et al.*, 2005). Recently, Mountzouris *et al.* (2009) reported that a multi-strain probiotic administered orally to broilers reduced *Salmonella* Enteritidis levels. This effect was associated with increased IgA and IgG antibodies against *S. enterica* at systemic and intestinal levels.

Even with extra care in handling food, pathogenic microorganisms can enter the human food chain and cause infections. Many of these infections are self-limiting when the immune system is working properly. However, some enteropathogens can cause serious problems to sensitive individuals such as young children and the elderly. Probiotics therefore appear to be an interesting strategy for improvement of health and prevention of gastrointestinal infections.

There are many studies where the beneficial effects of probiotics were reported against different infections. A randomized double-blind controlled study with infants of the age of 6 months showed that babies receiving follow-on formula supplemented with *L. fermentum* CECT5716 plus galactooligosaccharide (GOS) demonstrated a significant decrease (46% reduction) of the incidence rate of gastrointestinal infections compared to the control group that received the same formula supplemented with only GOS (Maldonado *et al.*, 2012). Another randomized double-blind study was carried out with shift workers and the results showed that daily consumption of a fermented milk containing *L. casei* DN-114 001 could reduce the risk of common infections in stressed individuals (Guillemard *et al.*, 2010).

Other probiotic microorganisms and products containing them were analyzed using randomized double-blind studies with different populations. The results showed an improvement in the groups which received probiotics, mainly in reduced infections of the respiratory tract, but no significant

differences were obtained considering gastrointestinal infections (Hojsak *et al.*, 2010; Taipale *et al.*, 2011). These results show that the probiotic effects are strain dependent and that it is not usual to obtain many beneficial effects from a unique probiotic strain. For this reason, the mixture of probiotic strains with different beneficial effects could be useful to increase the spectrum of action.

24.3 Prebiotics and synbiotics

Considering the importance of the balanced microbiota on gastrointestinal health, prebiotics are an alternative weapon in combating intestinal disorders. Prebiotics are defined as non-digestible food ingredients that stimulate the growth and/or activity of certain beneficial bacteria in the digestive system. They were first identified and named by Marcel Roberfroid in 1995. A more recent definition was made in 2007 in which a prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity, of the gastrointestinal microbiota, conferring benefits upon host well-being and health (Roberfroid, 2007). In that work, two particular fructo-oligosaccharides (FOS) fully meet all the criteria for prebiotic classifications: oligofructose and inulin. These prebiotic compounds are soluble and fermentable fibers referred to as fructans that reach the large intestine practically intact, are then hydrolyzed in the upper section of the intestine, and fermented by bacteria (Roberfroid *et al.*, 1998).

To enhance the therapeutic effects, the ability of probiotics to partially ferment prebiotics such as oligosaccharides and inulin in fermented milk has been studied. In fact, these compounds may also be added to foods in attempts to increase fiber ingestion and obtain low-calorie dairy products (Rodrigues *et al.*, 2011).

Oliveira *et al.* (2009) reported that in fermented milk prebiotics exert a protective effect and potentiate probiotic activity. In particular, the supplementation of yoghurt with inulin at low concentration significantly improves the growth and viability of *L. acidophilus* and *L. casei* during cold storage (Donkor *et al.*, 2007). In skimmed fermented milk by pure cultures of *Streptococcus thermophilus* and *Bifidobacterium lactis* or mixed cultures containing both (*S. thermophilus–B. lactis*), the presence of inulin improve quality and consistency. In particular, the time required by the combination of *S. thermophilus*, *B. lactis*, or *S. thermophilus–B. lactis* to complete fermentation was about 14.8 and 49% shorter than in the absence of this prebiotic, respectively. The inulin addition also enhanced the levels of lactic and acetic acids and volatile compounds, showing a positive synbiotic effect between pre- and probiotics. In addition, the *S. thermophilus–B. lactis* coculture showed final concentrations of both microorganisms about 15 and 38% higher than in their respective pure cultures, thus highlighting a clear synergistic effect between these microorganisms due to mutual interactions (Oliveira *et al.*, 2012b). Nevertheless, other lactobacilli also deserve attention due to their health-promoting effects (Ashraf & Shah, 2011).

The ability of probiotic microorganisms to ferment prebiotic ingredients may in fact be an especially important characteristic because the availability of carbohydrates that escape metabolism and adsorption in the small intestine has a major influence on the colon microbiota (Mattila-Sandholm *et al.*, 2002). Prebiotics are mainly used to selectively enhance lactobacilli and bifidobacteria and reduce colonization by pathogenic bacteria (Biggs and Parsons, 2008; Baurhoo *et al.*, 2009).

The combination of probiotics and prebiotics is called synbiotics and these have also been tested for their ability to prevent different infections in farm animals and in humans. Synbiotics are expected to be more beneficial than probiotics or prebiotics alone, due to synergistic effects. FOS and galacto-oligosaccharides (GOS) have been shown to promote growth of bifidobacteria in the colon. In fact, they are considered to have bifidogenic properties in humans. According to Gomes and Malcata (1999), numbers of *Bifidobacterium* strains can be increased either by continuous ingestion of bifidobacteria-containing

preparations or foods, or food can be supplemented with substrates (bifidogenic factors or prebiotics) that specifically promote the growth of endogenous bifidobacteria in the gut. The most feasible hypothesis regarding bifidogenic factors is that the prebiotic can stimulate the metabolism of bifidobacteria as it does in LAB, and the likely result is the increased level of fructose released from its partial hydrolysis mediated by inulinase, which may have been metabolized as an additional carbon and energy source (Mayo *et al.*, 2010).

The use of synbiotics in animals has been reported to assist in avoiding the abnormal growth of pathogenic bacteria in the intestine. For example, *Campylobacter jejuni* has emerged as a bacterial cause of foodborne gastroenteritis in humans, poultry and poultry products being the main source of this infection. An *in vivo* experiment was carried out to test the efficacy of the synbiotic composed of the microencapsulated *Bifidobacterium longum* subsp. *longum* PCB133 strain and a GOS using broiler chickens. Results demonstrated a significant increase of total bifidobacteria and a significant decrease of *C. jejuni* quantification after 2 weeks of treatment compared to the control group without specific administration (Baffoni *et al.*, 2012).

Synbiotics were also tested in childrens and adults. Special attention was paid to the incidence of infectious disease in infants switching from exclusive breast feeding to follow-on formula. In this sense, prebiotic- and probiotic-enriched formulas have been developed to mimic the beneficial effects of human milk on gut microbiota. Supplementation with synbiotic-enriched formula showed beneficial effects on the incidence of infectious disease and growth in infants compared to babies that received standard formula (Picaud *et al.*, 2010).

It is know that elderly people have variations in the intestinal microbiota with the development of potentially damaging bacteria compared to young adults. The gut microbiota provide a natural defense against invading microorganisms, and these alterations can be related to higher levels of infections in the elderly people. A randomized, double-blind, controlled trial was performed on healthy elderly volunteers using a synbiotic (*Bifidobacterium bifidum* BB-02 and *B. lactis* BL-01) together with an inulin-based prebiotic. The results reported that synbiotic consumption increased the size and diversity of protective bifidobacteria in feces, which are reduced in older people; these beneficial populations are also related to protective effects in these people (Bartosch *et al.*, 2005).

There are many other examples in the literature where the use of probiotics, prebiotics or synbiotics were reported in the prevention or treatment of infection diseases in humans and farm animals, remembering that the latter are at the beginning of the infection chain. The effects are strain specific for the probiotics and are different for each prebiotic and therefore also for each synbiotic. The different probiotics have demonstrated different effects against certain pathogens. Together with prebiotics, probiotics can reinforce these beneficial effects making the symbiotic preparation more potent against specific pathogens.

24.4   Production of bacteriocins by probiotic LAB

24.4.1   Production of antibacterial substances by LAB

Mechanisms of interaction between probiotic LAB and the host can be realized by several methods, including metabolization of toxic or undesirable chemical structures, stimulation of the immune response, aggregation between LAB and pathogenic bacteria, and by the production of antimicrobial substances that are active against other bacteria present in the gastrointestinal tract (GIT). LAB produce various antimicrobial substances during fermentation such as organic acids, hydrogen peroxide, carbon dioxide, diacetyl, low-molecular-weight antimicrobial substances, and bacteriocins (Blom and Mörtvedt, 1991). These specific antimicrobial compounds act as biopreservatives in food, with

records dating back to approximately 6000 BC (Pederson, 1971; De Vuyst and Vandamme, 1994). The antimicrobial substances are not produced for human convenience but rather for one bacterium to gain advantage over another while competing for the same energy source (Ouwehand and Vesterlund, 2004).

Various heterofermentative LAB produce equimolar amounts of lactic acid, acetic acid, ethanol, and CO_2 upon hexoses fermentation. Homofermentation results in the formation of lactic acid alone (Caplice and Fitzgerald, 1999). The antimicrobial effect of these organic acids formed during lactic acid fermentation is well known (Davidson, 1997). The organic acids, dissociated and undissociated, are believed to disrupt the mechanisms responsible for maintaining the membrane potential, thereby inhibiting active transport (Sheu *et al.*, 1972; Eklund, 1989; De Vuyst and Vandamme 1994).

LAB produce hydrogen peroxide in the presence of oxygen through the action of nicotinamide adenine dinucleotide (NADH) oxidases, flavoprotein-containing oxidases, and superoxide dismutase (Condon, 1987; Ouwehand and Vesterlund, 2004). LAB lack true catalase and it is therefore believed that hydrogen peroxide may accumulate and inhibit the growth of some microorganisms (Condon, 1987). However, it is argued that hydrogen peroxide is decomposed by flavoproteins, pseudocatalases and peroxidases *in vivo* and therefore does not accumulate to significant amounts (Nagy *et al.*, 1991; Fontaine *et al.*, 1996). Anaerobic environments can form due to some hydrogen-peroxide-producing reactions scavenging oxygen (Ouwehand and Vesterlund, 2004). Hydrogen peroxide production is important for the colonization of lactobacilli in the urogenital tract. This reduces the acquisition of gonorrhea, HIV and urinary tract infections (Vallor *et al.*, 2001).

Carbon dioxide is produced by heterolatic fermentation and contributes to an anaerobic environment that is toxic to various aerobic food microorganisms. Furthermore, carbon dioxide in itself has an antimicrobial activity (Lindgren and Dobrogosz, 1990). The mechanism involved in this activity is not known, but it is believed that carbon dioxide accumulates in the lipid bilayer due to the inhibition of enzymatic decarboxylations (King and Nagel, 1975), causing disfunction of membrane permeability (Lindgren and Dobrogosz, 1990). Low levels of CO_2 have been found to promote the growth of certain microorganisms, whereas high concentrations led to growth inhibition (Lindgren and Dobrogosz, 1990).

Diacetyl is produced from the fermentation of citrate and is responsible for the unique aroma and buttery flavor of various other fermented milk products (Lindgren and Dobrogosz, 1990; Cogan and Hill, 1993). Diacetyl is produced by many LAB, including the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus* (Jay, 1982). Gram-positive bacteria are less sensitive to its antimicrobial activity than Gram-negative bacteria, molds and yeast. The mechanism responsible for this activity is the action of diacetyl on the arginine-binding protein of Gram-negative bacteria, leading to interference with arginine utilization (Jay, 1982; Motlagh *et al.*, 1991; De Vuyst and Vandamme, 1994).

24.4.2 Production of bacteriocins by LAB

Several studies have focused on the production of low-molecular-weight antimicrobial substances by LAB (Reddy and Shahani, 1971; Hamdan and Mikolajcik, 1974; Shahani *et al.*, 1977a, b; Reddy *et al.*, 1983; Silva *et al.*, 1987). These substances share several characteristics in addition to having a low molecular weight, such as being active at a low pH, soluble in acetone, thermo-stable, and displaying a broad spectrum of activity (Axelsson, 1990). However, more in-depth studies need to be done to gain detailed information on these substances. So far, three low-molecular-weight antimicrobial substances have been properly characterized: Reuterin and Reutericyclin, both produced by *L. reuteri*; and 2-Pyrrolidone-5-carboxylic Acid, produced by *L. casei* subsp. *casei*, *L. casei* subsp.

pseudoplantarum and *Streptococcus bovis* (Chen and Russell, 1989; Huttunen *et al.*, 1995).

LAB are known for their production of bacteriocins or bacteriocin-like peptides (Todorov, 2009). Bacteriocins of LAB are defined as ribosomally synthesized proteins or protein complexes, usually antagonistic to genetically closely related organisms (Nes and Johnsborg, 2004). They are generally low-molecular-weight proteins that gain entry into target cells by binding to cell surface receptors. Their bactericidal mechanisms vary and may include pore formation, degradation of cellular DNA, disruption through specific cleavage of 16S rDNA, and inhibition of peptidoglycan synthesis (James *et al.*, 1991; Heu *et al.*, 2001).

Bacteriocins are divided into four main classes. Class I peptides, known as lantibiotics (<5 kDa), are post-translationally modified and contain lanthionine and β-methyl-lanthionine. Class II bacteriocins are small (<10 kDa), heat-stable, cationic, hydrophobic, and membrane-active peptides (De Vuyst and Vandamme, 1994). Bacteriocins with the highly conserved N-terminal amino acid sequence YGNGVXaaC (Tyr-Gly-Asn-Gly-Val-Xaa-Cys), non-polar amino acids, one or more disulfide bridges and activity against *Listeria* spp. are grouped in class IIa (Eijsink *et al.*, 1998; Ennahar *et al.*, 2000). Bacteriocins that function in pairs, usually as two distinct peptides, are grouped in class IIb (Ennahar *et al.*, 2000). Thiol-activated bacteriocins that rely on a *sec*-dependent secretion mechanism are categorized into class IIc (Héchard and Sahl, 2002). Class III bacteriocins are large (>30 kDa) heat-labile proteins. It has therefore been proposed that this class may include bacteriolytic enzymes such as hemolysins and muramidases, which are able to imitate the physiological activities of bacteriocins (Jack *et al.*, 1994). This group is the least well characterized and so far has only been isolated from the genus *Lactobacillus* (Klaenhammer, 1993). Class IV are the bacteriocins with a complex structure and glyco- and/ or lipid moieties. Class I and II bacteriocins are considered the most important due to potential commercial applications.

Apart from competition for binding sites and production of hydrogen peroxide, bacteriocins (antimicrobial peptides) may play a key role in the mode of action of probiotic LAB (Velraeds *et al.*, 1998; Boris and Barbes, 2000; Lepargneur and Rousseau, 2002; Reid and Burton, 2002). Bacteriocin production is not a compulsory characteristic of probiotic LAB, but gives them an advantage in the interaction with non-desirable bacteria from the GIT. Although the role of bacteriocins and their significance in controlling the proliferation of pathogenic bacteria in the intestinal tract is questionable (Brink *et al.*, 2006), recent reports on bacteriocins active against Gram-negative bacteria (Ivanova *et al.*, 1998; Messi *et al.*, 2001; Caridi, 2002; Todorov and Dicks, 2005a) have renewed interest in these peptides and their interaction with intestinal pathogens.

24.4.3 Production of bacteriocins by LAB present in fermented cereals

It has also been shown that several bacteriocinogenic strains have potential probiotic properties (van Reenen *et al.*, 1998; Todorov and Dicks, 2005a, c, 2006, 2008; Todorov *et al.*, 2005, 2006, 2007, 2011, 2012; Powell *et al.*, 2007; Botes *et al.*, 2008; Knoetze *et al.*, 2008).

Countries of the Balkan region in Europe are famous for the production of food and beverages fermented with LAB. Boza is one such traditional drink, produced through the fermentation of different cereals by yeast and lactic acid bacteria. However, only a few papers have been published on the microbial composition of boza; most of the LAB that have been isolated belong to the genera *Lactobacillus*, *Lactococcus*, and *Leuconostoc* (Hancioglu and Karapinar, 1997; Gotcheva *et al.*, 2000; Kabadjova *et al.*, 2000; Zorba *et al.*, 2003; Botes *et al.*, 2007). Ivanova *et al.* (2000), Kabadjova *et al.* (2000), Todorov and Dicks (2004, 2005b, 2006), Von Mollendorff *et al.* (2006), and Todorov (2010) reported on strains isolated

from boza with activity against Gram-positive bacteria including *Listeria* spp., and Gram-negative bacteria, including *Escherichia coli*.

However, even if the numerous bacteriocinogenic LAB isolated from boza have been reported, to our knowledge only one study has addressed the potential probiotic properties of these isolates. Todorov *et al.* (2007) isolated a number of LAB with probiotic properties from boza. *L. plantarum* ST194BZ, ST441BZ, and ST664BZ, *L. paracasei* ST242BZ and ST284BZ, *L. rhamnosus* ST461BZ and ST462BZ and *L. pentosus* ST712BZ, previously reported to be bacterioin producers (Todorov and Dicks, 2006). These survived low pH conditions (pH 3.0), grew well at pH 9.0, and were not affected by the presence of 0.3% (w/v) oxbile. Cytotoxicity levels of the bacteriocins, expressed as CC_{50}, ranged from $38 \mu g\,mL^{-1}$ for bacteriocin ST194BZ to $3776 \mu g\,mL^{-1}$ for bacteriocin ST284BZ. Bacteriocins ST284BZ, ST461BZ and ST462BZ were the least cytotoxic. Bacteriocin ST284BZ revealed high activity ($EC_{50} = 735 \mu g\,mL^{-1}$) against the HSV-1 virus that causes encephalitis and orofacial and genital lesions. Growth of *Mycobacterium tuberculosis* was repressed (69%) after 5 days of incubation in the presence of bacteriocin ST194BZ. Various levels of auto (self) aggregation between the probiotic bacteria and co-aggregation with *Listeria innocua* LMG 13568 were observed. Adhesion of *L. plantarum* ST194BZ, ST441BZ, and ST664BZ, *L. paracasei* ST242BZ and ST284BZ, *L. rhamnosus* ST461BZ and ST462BZ and *L. pentosus* ST712BZ to HT-29 cells ranged from 18% to 22%, which is similar to that reported for *L. rhamnosus* GG, a commercial probiotic. Adherence of strains *L. plantarum* ST194BZ, *L. paracasei* ST242BZ, and *L. pentosus* ST712BZ to Caco-2 cells ranged between 7.0% and 9.0% and is similar to values reported for *L. rhamnosus* GG. High hydrophobicity readings were recorded for most of the probiotic strains. *L. pentosus* ST712BZ revealed only 38% hydrophobicity, but 63% of cells adhered to HT-29 cells, compared to 32% adherence recorded for *L. rhamnosus* GG. Growth of *L. plantarum* ST194BZ, ST441BZ, and

ST664BZ, *L. paracasei* ST242BZ and ST284BZ, *L. rhamnosus* ST461BZ and ST462BZ, and *L. pentosus* ST712BZ were inhibited by only 7 of the 24 medicaments tested.

24.4.4 Production of bacteriocins by LAB present in other fermented foods

Todorov and Dicks (2008) reported eight bacteriocins produced by LAB isolated from fermented olives, molasses, sorghum beer, and kefir with activity against Gram-positive and Gram-negative bacteria and studied the survival of the strains in the presence of low pH and elevated levels of oxbile, adhesion to Caco-2 cell lines, growth in the presence of antibiotics and commercially available medicaments, and the presence of genes encoding adhesion surface proteins. Studied strains were *L. plantarum* ST26MS and ST28MS isolated from molasses (Todorov and Dicks, 2005a), *L. plantarum* ST23LD, *Enterococcus faecium* ST311LD, *Leuconostoc mesenteroides* subsp. *mesenteroides* ST33LD, and *L. plantarum* ST341LD isolated from spoiled fermented black olives (Todorov and Dicks, 2005b), *L. plantarum* 423 isolated from beer (van Reenen *et al.*, 1998), and *L. plantarum* ST8KF isolated from kefir (Powell *et al.*, 2007).

The above-mentioned strains (Todorov and Dicks, 2008) have been investigated for growth at low pH and oxbile resistance. Good growth was recorded in de Man, Rogosa and Sharpe (MRS) broth supplemented with 0.3% (w/v) oxbile. *L. plantarum* ST28MS and ST26MS, *E. faecium* ST311LD, and *L. mesenteroides* subsp. *mesenteroides* ST33LD grew well in the presence of 0.6% (w/v) oxbile. All eight strains grew well in MRS broth adjusted to pH 7.0. Good growth of *E. faecium* ST311LD, *L. mesenteroides* subsp. *mesenteroides* ST33LD, and *L. plantarum* 423 was recorded at pH 4.0. In addition, aggregation properties have been tested. Auto and co-aggregation properties are important features for the probiotic LAB, but needs to be investigated and discussed in combination with antimicrobial properties of the tested strains. High aggregation

properties can lead to good biofilm formation ability. When LAB produces antimicrobial compounds against co-aggregation partners, this facilitates their exclusion from the biofilm. In contrast, the low levels of co-aggregation with pathogens may play an important role in preventing the formation of biofilms, and in this way eliminate the pathogens from the GIT. In case of *L. plantarum* ST26MS and ST28MS, *L. plantarum* ST23LD, *E. faecium* ST311LD, *L. mesenteroides* subsp. *mesenteroides* ST33LD, and *L. plantarum* ST341LD, *L. plantarum* 423 and *L. plantarum* ST8KF auto cell-aggregation ranged from 74.3% for *L. plantarum* ST23LD to 95.4% for *L. plantarum* ST28MS. Different levels of co-aggregation were recorded between the eight strains and *E. faecium* HKLHS, *L. sakei* DSM 20017, *Lactococcus lactis* subsp. *lactis* HV219, *L. innocua* LMG 13568 and UWC N27, and *Listeria ivanovii* susbp. *ivanovii* ATCC 19119 (Todorov and Dicks, 2008).

24.4.5 Effect of commercial drugs on bacteriocin production by LAB

Another important point is interaction between commercial drugs and probiotic LAB. The mechanism of the inhibitory effect against probiotic LAB and other GIT-related bacteria needs to be related to the chemical composition of drugs. A simple recommendation would be not to apply a drug with an inhibitory effect to the probiotic/beneficial LAB at the same time, since the drug will have a negative effect on the probiotic cells and result in decreased viability.

The application of drugs together with starter and probiotic/beneficial cultures needs to be reconsidered regarding the possibility of a negative interaction. The minimum inhibitory concentration (MIC) of the drug on the survival and growth of beneficial bacteria is an important parameter. It is important to keep in mind that some drugs are not taken by the patient permanently. The daily dose for this drug needs to be linked with the MIC against probiotic LAB. Especially important are drugs used in the treatment of chronic diseases. Growth of *L. plantarum*

423, ST8KF, ST23LD, ST26MS, ST28MS, and ST341LD, *E. faecium* ST311LD, and *L. mesenteroides* subsp. *mesenteroides* ST33LD were inhibited by several antibiotics and anti-inflammatory medicaments containing ibuprofen, hydrochlorothiaziden, and thioridazine hydrochlorid (Todorov and Dicks, 2008). Sodium diclofenac inhibited the growth of *L. plantarum* ST8KF and ST341LD, *E. faecium* ST311LD, and *L. mesenteroides* subsp. *mesenteroides* ST33LD. Dimenhydrinate inhibited the growth of only *L. plantarum* ST8KF (Todorov and Dicks, 2008).

Adherence of *L. plantarum* 423, ST23LD, ST26MS, ST8KF, ST28MS, and ST341LD, *E. faecium* ST311LD, and *L. mesenteroides* subsp. *mesenteroides* ST33LD to Caco2 cells ranged from 8.0% to 1.3%. However, a disputable point is whether the Caco2 cells are an appropriate model to study probiotic properties. In order to investigate *in vitro* interactions between probiotic bacteria and the intestinal mucosa, several cell lines and culturing models have been used (Botić *et al.*, 2007; Yang *et al.*, 2007; Ewaschuk *et al.*, 2008; Zoumpopoulou *et al.*, 2009). The human colon tumorigenic cell lines Caco2, T84, and HT-29 have been widely utilized for bacterial adhesion assays and mechanistic studies, but are not an appropriate model for pathogen–host interactions because they do not derive from the small intestine, have a tumorigenic phenotype distinct from that of normal gut epithelia, express modified surface glycoconjugates, and are much more glycosylated than normal cells. These latter considerations are important due to the increasing significance of cell-culture-based assays that mimic the *in vivo* environment (Klingberg *et al.*, 2005; Tremblay *et al.*, 2007).

These problems of accuracy can be overcome by the use of H4, PSIc1, and CLAB cell lines; among these, the first was obtained from the small intestinal tissue of a human fetus at 20–22 weeks of gestation (Sanderson *et al.*, 1995) and further subcloned. Moreover, these cell lines are not of tumor origin so are therefore a better *in vitro* model to study the mechanisms of gut interaction than human tumorigenic cell lines. The cell line H4 is a non-cancerogenic

cell line, positive for epithelial markers cytokeratins, alkaline phosphatase, and laminin. Cells polarize when growing on microporous inserts and develop micovilli and transepithelial resistance (TER) up to 1000 ohms. The H4 cell line obtained from pigs is the closest to humans in terms of genome, organ, development, anatomy, physiology, and metabolism of the intestinal tract (Brown and Timmermans, 2004), disease progression (Lunney, 2007), and for intestine–microbe interactions (Pipenbaher *et al.*, 2009; Maragkoudakis *et al.*, 2010).

All eight strains (*L. plantarum* 423, ST8KF, ST23LD, ST26MS, ST28MS, and ST341LD, *E. faecium* ST311LD, and *L. mesenteroides* subsp. *mesenteroides* ST33LD) generated positive results for the presence of *Mub*, *MapA* and *EF-Tu* genes, as determined by amplification with gene-specific primers (Todorov and Dicks, 2008). The expression of mucus adhesion proteins, such as those encoded by the *mub* and *mapA* genes and of GTP-binding EF-Tu protein, has been shown to be critical in adhesion of probiotic strains to human intestine cells (Ramiah *et al.*, 2007). Most probably the presence of these genes is species dependent and not related to the ecological origin of the strain(s). However, these same genes were recorded in other *L. plantarum* strains isolated from plants (Ramiah *et al.*, 2007). The presence of these adhesion genes in this *L. plantarum* strain is not surprising due to the high adhesive properties of this microbial species. Whole genome sequence data of *L. plantarum* WCFS1, a human isolate, revealed the presence of 223 extracellular proteins, most of them involved in the adhesion of the cell to its environment (Kleerebezem *et al.*, 2003). The domain composition of *L. plantarum* WCFS1 proteins predicted to be associated with adhesion indicated the presence of seven proteins involved with mucus. Some of these possible mucus-binding proteins contain the Mub domain, a trait unique to LAB (Boekhorst *et al.*, 2006). The presence of these adhesive proteins in lactic acid bacteria is imperative for probiotic strains to colonize the GIT.

24.4.6 Antibiotic resistance in bacteriocins producing LAB

Antibiotic resistance is a fundamental characteristic for the safety evaluation of probiotic LAB. Acquired resistance, located on plasmids and transposons, is the main concern (Giraffa, 2002; Franz *et al.*, 2005). Intrinsic resistance to some antibiotics is a feature commonly found among *Enterococci* strains isolated from foods, as reported by Franz *et al.* (2005). However, although much attention has been paid to the *Enterococcus* spp., we need to be concerned about the antibiotic resistance of all LAB species in their application as probiotics or functional starter cultures in the food industry.

Transfer of resistance against antimicrobial substances is an essential mechanism of LAB if they are able to adapt and survive in specific environments. Resistance mechanisms include enzymatic inactivation of the antibiotics, restricted import of antibiotics, and active export of antibiotics. Target modifications may therefore be better focus points in future research. Resistance may be inherent to a bacterial genus or species, but may also be acquired through exchange of genetic material, mutations and the incorporation of new genes (Ammor *et al.*, 2007). Teuber *et al.* (1999) and Salyers *et al.* (2004) suggested that starter cultures and probiotics may serve as vectors in the transfer of antibiotic-resistant genes. Such transfer was documented in other bacterial groups by Levy and Marshall (2004) and Salyers *et al.* (2004).

Bacteriocin production by *L. plantarum* ST16Pa isolated from papaya fruit have been described by Todorov *et al.* (2011). Strain ST16PA was identified as *L. plantarum* based on biochemical tests, PCR with species-specific primers, and 16S rDNA sequencing and shown to produce a 6.5 kDa bacteriocin, active against different species from genera *Enterobacter*, *Enterococcus*, *Lactobacillus*, *Pseudomonas*, *Streptococcus*, and *Staphylococcus* and different serotypes of *Listeria* spp. However, it is important to highlight activity against pathogenic bacteria which will give an advantage for this strain if applied as a probiotic. The antimicrobial peptide produced by

L. plantarum ST16Pa was inactivated by proteolytic enzymes, but not when treated with α-amylase, catalase, lipase, Triton X-100, SDS, Tween 20, Tween 80, urea, NaCl, and EDTA, a characteristic described for several bacteriocins. However, the presence of 1% Triton X-114 deactivates the bacteriocin. No change in activity was recorded after 2 hr at pH values between 2.0 and 12.0, and after treatment at 100 °C for 120 min or 121 °C for 20 min. The mode of activity against *L. sakei* ATCC 15521, *Enterococcus faecalis* ATCC 19443, and *L. innocua* 2030C was bactericidal, resulting in cell lysis and enzyme leakage. No significant differences in cell growth and bacteriocin production were observed when *L. plantarum* ST16Pa was cultured in MRS broth at 26 °C and 30 °C for 24 h ($25,600 \, AU \, mL^{-1}$). However, even though *L. plantarum* ST16PA grows well in MRS broth at 15 °C and 37 °C, a reduction of bacteriocin production was observed ($400 \, AU \, mL^{-1}$ and $1600 \, AU \, mL^{-1}$, respectively). In addition, the effect of MRS medium components, different initial pH, and additions of glycerol or vitamins to the media on bacteriocin ST16Pa production was studied (Todorov *et al.*, 2011).

The antimicrobial peptide produced by *L. plantarum* ST16PA adsorbs ($400 \, AU \, mL^{-1}$) producer cells. However, bacteriocin ST16Pa was adsorbed at 50% to cells of *L. innocua* 2030C and at 75% to *L. sakei* ATCC 15521 and *E. faecalis* ATCC 19433 in experiments conducted at 30 °C and pH 6.5. Adsorption of bacteriocin ST16Pa to target cells at different temperatures and pH and in the presence of potassium sorbate, sodium nitrate, sodium chloride, ascorbic acid, Tween 80, and Tween 20 were also studied (Todorov *et al.*, 2011).

In addition to previous work (Todorov *et al.*, 2011), the potential probiotic and beneficial properties of *L. plantarum* ST16Pa were investigated (Todorov *et al.*, 2012). The ability to produce antilisterial bacteriocins by LAB can be explored by the food industry as a tool to increase the safety of foods. Furthermore, probiotic activity of bacteriogenic LAB brings extra advantages to these strains, as they can confer health benefits

to the consumer. Beneficial effects depend on the ability of the probiotic strains to maintain viability in the food during shelf-life and to survive the natural defenses of the host and multiply in the gastrointestinal tract (GIT).

Todorov *et al.* (2012) evaluated the probiotic potential of a bacteriocinogenic *L. plantarum* ST16Pa isolated from papaya fruit and studied the effect of encapsulation in alginate on survival in conditions simulating the human GIT. Good growth of *L. plantarum* ST16Pa was recorded in MRS broth with initial pH values between 5.0 and 9.0 as well as the ability to survive at pH values of 4.0, 11.0, and 13.0. *L. plantarum* ST16Pa grew well in the presence of oxbile at concentrations ranging from 0.2% to 3.0%. The level of auto-aggregation was 37%, and various degrees of co-aggregation were observed with different strains of *L. plantarum*, *Enterococcus* spp., *L. sakei*, and *Listeria*, which are important features for probiotic activity. Growth was affected negatively by several medicaments used for human therapy, mainly anti-inflammatory drugs and antibiotics. Adhesion to Caco2 cells was within the range reported for other probiotic strains, and PCR analysis indicated that the strain harbored the adhesion genes *mapA*, *mub* and *EF-Tu*. Encapsulation in 2%, 3% and 4% alginate protected the cells from exposure to 1% or 2% oxbile added to MRS broth. Studies in a model simulating the transit through the GIT indicated that encapsulated cells were protected from the acidic conditions in the stomach, but were less resistant when in conditions simulating the duodenum, jejunum, ileum, and first section of the colon (Todorov *et al.*, 2012).

It has also been shown (Todorov *et al.*, 2012) that there are several drugs that reduce the growth of *L. plantarum* ST16Pa, especially non-steroidal anti-inflammatory drugs that contain potassium diclofenac or ibuprofen arginine, promethazine hydrochloride (antihistaminic), orphenadrine citrate, and sodium metamizole (analgesic), paroxetine (antidepressant) and amiodarone (antiarrhythmic). These results emphasize that the application of probiotic strains needs to be take into account the types

of medicaments being used. The negative effect of sodium diclofenac on growth of potential probiotic strains was also observed in other studies (Todorov *et al.*, 2007, 2008; Botes *et al.*, 2008; Todorov and Dicks, 2008; Carvalho *et al.*, 2009). Dimenhydrinate inhibited the growth of *L. rhamnosus* ST462BZ and *L. plantarum* ST664BZ (Todorov *et al.*, 2008).

The majority of the antibiotics tested in the study of Todorov *et al.* (2012) inhibited the growth of *L. plantarum* ST16Pa, highlighting that the strain presents a low risk for transfer of antibiotic resistance. The strain was resistant to amicacin, tobramicin, vancomycin, oxacillin, kanamycin, metronidazole, and nalidixic acid. Resistance of potential probiotic LAB to antibiotics is a controversial subject, as these strains may be reservoirs of antibiotic resistance genes and can be transferred horizontally to others in the human GIT (Dicks *et al.*, 2011). Resistance may be inherent to a bacterial genus or species, but may also be acquired through exchange of genetic material, mutations, and the incorporation of new genes (Teuber, 1999; Levy and Marshall, 2004; Salyers *et al.*, 2004; Ammor *et al.*, 2007).

A similar study regarding the bacteriocin production and potential probiotic properties have been reported for *L. lactis* subps. *lactis* HV219 isolated from vaginal secretions (Todorov *et al.*, 2006, 2007). Bacteriocin HV219, produced by *L. lactis* subsp. *lactis* HV219, is active against Gram-positive and Gram-negative bacteria. Its mode of activity was bacteriolytic, as confirmed by atomic force microscopy. Bacteriocin has been expressed in highest levels at 37 °C (Todorov *et al.*, 2006). In addition to the bacteriocinogenic properties, a potential application as vaginal probiotic has been shown for *L. lactis* subsp. *lactis* HV219 (Todorov *et al.*, 2007). *L. lactis* subsp. *lactis* HV219 demonstrated a good resistance to acids, bile, inflammatory drugs, and spermicids. Adsorption of bacteriocin HV219 pathogenic bacteria was influenced by pH, temperature, surfactants, and salts, allowing the bacteriocin to be more effective in simulating bacterial vaginosis. *L. lactis* subsp. *lactis* HV219 was sensitive to most tested antibiotics (Todorov *et al.*, 2007).

A good example of *Enterococcus* spp. bacteriocin producer and potential probiotic is *Enterococcus mundtii* ST4SA (Todorov *et al.*, 2005; Botes *et al.*, 2008; Knoetze *et al.*, 2008). *E. mundtii* ST4SA (ST4V) was isolated from soya bean and characterized as a bacteriocin producer (Todorov *et al.*, 2005; Knoetze *et al.*, 2008). Peptide ST4SA, produced by *E. mundtii* ST4SA, inhibits the growth of *Acinetobacter baumannii*, *E. faecalis*, *E. faecium*, *Staphylococcus aureus*, *Streptococcus pneumonia*, and Gram-positive bacteria isolated from patients diagnosed with middle ear infections. The peptide adsorbed at a level of 94% to *S. pneumoniae* 40, *Pseudomonas aeruginosa* 25, and *E. faecium* HKLHS. Low concentrations of peptide ST4SA (51,200 AU mL^{-1}) caused DNA and enzyme leakage from target cells, while 1,638,400 AU mL^{-1} caused cell lysis. No decrease in antimicrobial activity was observed when tested on a solid medium with human blood as base.

Peptide ST4SA revealed a similar level of activity compared to tetracycline (30 µg), but much higher activity compared to nasal sprays, aminoglycosides, cephalosporins, fluoroquinolones, lincosamides, macrolides, nitroimidazole, penicillin, quinolones, sulphonamides, chloramphenicol, furazolidone, fusidic acid, rifampicin, trimethoprim, trimethoprim/sulfamethoxazole, and vancomycin when tested *in vitro*. Peptide ST4SA dissipates the proton-motive force and may be used in the treatment of multidrug-resistant strains where antibiotics are excluded from cells by efflux pumps dependent on the membrane proton gradient (Knoetze *et al.*, 2008).

It has also been shown that this antimicrobial peptide has anti-viral properties (Todorov *et al.*, 2005). The peptide inactivated the herpes simplex viruses HSV-1 (strain F) and HSV-2 (strain G), a polio virus (PV3, strain Sabin), and a measles virus (strain MV/BRAZIL/001/91, an attenuated strain of MV). MV, HSV-1, and HSV-2 were 95.5–99.9% inactivated by peptide ST4V at 400 µg mL^{-1}. Monkey kidney Vero cells were not inactivated, even at four times the level peptide ST4V displayed antiviral activity, indicating that the effect was not due to cytotoxicity (Todorov *et al.*, 2005).

In the study of Botes *et al.* (2008) *E. mundtii* ST4SA were investigated as a potential probiotic. *E. mundtii* ST4SA survived intestinal conditions simulated in a gastro-intestinal model (GIM) with infant milk formulations as substrate and prevented the growth of *L. monocytogenes* ScottA. The strains are inhibited by the antibiotics amoxicillin, cefadroxil, roxithromycin, and doxycycline, anti-inflammatory medicaments containing meloxicam, ibuprofen, and sodium diclofenac, and analgesics containing paracetamol, codeine phosphate, and promethazine. However, genes encoding AS, cytolysin and non-cytolysin (β-hemolysin III) were amplified from the genome of strain ST4SA. Survival of strain ST4SA improved when used as combined cultures with *L. plantarum* 423, a bacteriocin producer (van Reenen *et al.*, 1998), in the GIM and compared well with the survival of commercially available probiotics subjected to the same conditions (Botes *et al.*, 2008).

Todorov *et al.* (2009) applied *E. mundtii* ST4SA (ST4V) as a non-starter co-culture in the preparation of boza with the aim of increasing the safety of this traditional cereal-based beverage regarding bacteriocin production and of using boza as a vector for delivery of the probiotic strain. Boza was prepared according to the traditional recipe and inoculated with *E. mundtii* ST4V (a potential probiotic and bacteriocin-producing strain). Commercially produced boza, *Saccharomyces cerevisiae*, and a combination of strain *E. mundtii* ST4V and *S. cerevisiae* was also used. Fermentation was carried out at 37 °C for 3 hr. The organoleptic properties of fermented products were evaluated by a qualified taste panel. No significant differences in rheological properties were observed, suggesting that *E. mundtii* ST4V had no effect on the quality of the final product.

Microbial cell numbers remained relatively unchanged during one week of storage, the normal shelf life of this product. The preservative properties of bacteriocin ST4V were evaluated by contaminating boza with *L. sakei* DSM 20017, a strain sensitive to the bacteriocin produced by *E. mundtii* ST4V. Changes in microbial populations were monitored by using classical microbiological methods, PCR with species-specific primers, and denaturing gradient gel electrophoresis (DGGE). Adsorption of bacteriocin ST4V to target cells is pH-dependent, with the highest adsorption (88%) recorded at pH values of 8.0 and 10.0.

Maximum adsorption of bacteriocin ST4V (75%) to *E. faecalis* and *L. innocua* was recorded at 25–37 °C. Growth of *E. mundtii* ST4V was inhibited only by a few antibiotics and anti-inflammatory medicaments, suggesting that the strain may be used as a probiotic by individuals receiving medical treatment (Todorov *et al.*, 2009). The conclusion from this study was that *E. mundtii* ST4V may be used as a non-starter co-culture in preparation of boza and will have a positive effect on the safety of this beverage. However, *E. mundtii* ST4V remains present during the storage period of the drink and in this way boza may be used as a vector for delivery for this potential probioitc strain. It is important to highlight that most of the food matrices used for the delivery of probiotics are milk products. Sensorial analysis has demonstrated that consumers cannot detect organoleptical differences related to the presence of *E. mundtii* ST4SA in boza (Todorov *et al.*, 2009).

In conclusion, bacteriocin production is an advantage for probiotic LAB, giving them a better chance of survival in the GIT while competing with other bacteria present in the same ecological environment. Their ability to prevent the growth of certain pathogens is also interesting from a technological and, more importantly, public health point of view, and could be useful tool to decrease the incidence of foodborne diseases.

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25

Predictive Microbiology: A Valuable Tool in Food Safety and Microbiological Risk Assessments

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Summary

Foodborne pathogens represent a major concern for the food industry, which needs to ensure that products are safe for consumers. Predictive microbiology is a multidisciplinary area of food microbiology, which is devoted to studying and predicting, by means of mathematical models, the effects of extrinsic factors (temperature, salt, preservatives, etc.) on microbial behaviour in foods. The present chapter is an overview of the successive steps to follow for the successful development and implementation of a predictive model, as well as the most common types of secondary models used in food microbiology. The chapter continues with a general review of the most powerful predictive microbiological tools (tertiary models) that are currently available on the worldwide web for foodborne pathogens, and concludes by commenting on the direct implications and applications of predictive microbiology in food safety and microbial risk assessments.

25.1 Introduction

Many types of beverages and foods are produced industrially through the favourable action of microorganisms (ICMSF, 2005; Querol and Fleet, 2006). However, microorganisms can also represent an important risk for consumers. Pathogen species such as *Salmonella* T, *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes* or *Clostridium botulinum*, among others, cause thousands of outbreaks of food diseases each year around the world due to poorly processed foods, both in developing and developed countries (Much *et al.*, 2009; Minami *et al.*, 2010; Newell *et al.*, 2010). Unwanted microbial growth in a food processing environment can therefore be disastrous. The economic and social consequences of microorganisms in foods depend not only on the species present but, most importantly, on their quantitative populations. Populations in foods are not static and can change both qualitatively and quantitatively throughout the production or packaging. It is the number of microbial cells that ultimately determines whether or not the product will cause an outbreak of disease (Fleet, 1999).

From a public health point of view, the importance of the presence of pathogenic microorganisms in foods is indisputable, by just glazing the statistics of human cases. In 2010, 5262 outbreaks were reported in Europe (EFSA, 2012) and about 19,089 of outbreaks in the USA (CDC, 2011). EFSA in Europe reported that *Salmonella*, viruses, *Campylobacter* and bacterial toxins caused most of the foodborne outbreaks and the main food sources were eggs, mixed or buffet meals and vegetables (EFSA, 2012).

The socioeconomic repercussions of the costs of foodborne illnesses are not always easy to deal with. There have been several approaches of this cost estimation (Flint *et al.*, 2005; Kuchenmuller *et al.*, 2009; Scallan *et al.*, 2011). In a recent US study, Hoffman *et al.* (2012) expanded the number of factors to take into account this fact, and estimated that the economic cost of foodborne outbreaks was about USD 14 billion. This was attributed to the 14 most common pathogens.

This list is headed by non-typhoidal *Salmonella enterica*, *Toxoplasma gondii*, *L. monocytogenes*, norovirus and *Campylobacter spp*. The same authors described how most of the reported cases are associated with poultry products, pork products and 'complex foods' responsible for 60% of the outbreak costs (Batz *et al.*, 2012).

Salmonella is recognized worldwide as one of the most common pathogens causing gastroenteritis after consumption of contaminated food (Batz *et al.*, 2012; EFSA, 2012). The disease is characterized by the presence of acute fever, abdominal cramps, nausea and vomiting. In the European Union, 99,020 cases were reported in humans in 2010 (EFSA, 2012). In the US, 1.4 million people per year are estimated to be infected with salmonellosis (Scallan *et al.*, 2011). Likewise, in Asia *Salmonella* causes more than 37,600 annual deaths (Majowicz *et al.*, 2010).

T. gondii infects animals and humans, felines being the primary host and principal source of infection because of the excretion of the infective oocyst to the environment. *T. gondii* is one of three pathogens, together with *Salmonella* and *Listeria*, which account for more than 75% of deaths due to foodborne disease in the US and is strongly associated with undercooked or raw pork and beef meat (Batz *et al.*, 2012). It can also be found in lamb and poultry. This infection causes flu, swollen lymph nodes including muscle aches and pains, stillbirth, severe mental retardation, loss of vision and congenital problems in human health infants.

L. monocytogenes is a microorganism able to grow at refrigeration temperatures in contaminated foods (Koseki and Isobe, 2005). It is responsible for opportunistic infections, with the main risk population sectors being pregnant women, infants and the elderly. The symptoms are meningitis, encephalitis, bacteremia or stillbirth. The mortality rate in adults is high ranging from 33% to 62%. In the European Union there were 1601 reported cases in humans in 2010 (EFSA, 2012), while in the US this pathogen is responsible for about 1500 illnesses and 255 annual deaths (Scallan *et al.*, 2011). Due to its ubiquitous nature, *L. monocytogenes* has been

isolated from ready-to-eat foods such as raw milk cheeses, cold smoked fish, fermented meats, prepared salads (Ray, 2004) and processing environments (Miettinen *et al.*, 1999; Samelis and Metaxopoulos, 1999; Kabuki *et al.*, 2004).

Norovirus, a member of the Caliciviridae family, is the major cause of acute gastroenteritis worldwide. The symptoms of vomiting, diarrhoea, abdominal pain and slight fever arise after an incubation time of 2 days and usually last only 3 days. Most affected people do not require medical attention, but immunocompromised patients may require hospitalization for rehydration therapy. Undercooked shellfish is the food mostly associated with this agent.

Bacteria of the genus *Campylobacter* spp. are often associated with several cases of gastroenteritis, especially in children. In 2008 in the European Union, there were a total of 212,064 cases of campylobacteriosis in humans, describing an upward trend (EFSA, 2010). Among the symptoms presented, the appearance of bloody diarrhoea, abdominal pain, fever and headache are catalogued as the most common. One of the main transmission routes is related to undercooked or raw foods, or by cross-contamination of food caused by the use of cutting boards, knives and other kitchen utensils (Gill and Harris, 1984). Chicken meat is one of the foods involved, even though it is evidenced in other foods such as raw milk, dairy products, fish and fresh vegetables.

E. coli O157: H7 is characterized by producing infectious processes that cause severe complications such as hemorrhagic colitis and abdominal cramps. In addition, the disease produces hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura boxes. Ingestion of contaminated water and food (such as beef, pork, chicken, burgers, fresh vegetables or milk) are the most common transmission routes. In 2010, this serotype was responsible for 4000 cases of foodborne diseases in the European Union. This represented 12% of that produced in 2009, where children of 0–4 years (1161 cases) and 5–14 years (>40 cases) were the most affected (EFSA, 2012). Globally, the prevalence of infections with other non-O157 enterohemorragic serogroups are greatly underestimated in Europe, Australia and Latin America (Montville and Matthews, 2009). Recent evidence was the large outbreak reported in Germany in 2011, after consumption of sprouts contaminated with *E. coli* O104:H4 (EFSA, 2011).

For all these reasons, it is essential to control the microbial life cycle in food ecosystems, favouring the growth of desirable microorganisms and inhibiting spoilage and pathogens. There are certain factors, so-called extrinsic factors, which affect microorganism response in foods. The most important include temperature, water activity, pH or diverse types of preservatives (sorbate, benzoate, acetic acid, sulphites, etc.). The effects of these extrinsic factors on microorganism response have been widely studied by food microbiologists, especially for foodborne pathogens (ICMSF, 1996), since they can be easily modified and controlled by the food industry during processing. Rarely a single stress present in food environments, and microbial cells are simultaneously exposed to a combination of stresses (hurdle effect). The effect of the combined stresses on growth and survival may be additive or synergistic, which has a significantly greater influence on microorganisms (Leistner and Gorris, 1995).

The study of all these effects on microbial response in food ecosystems is the basis of predictive and risk assessment strategies. Reliable, confident decisions concerning public health require quantitative ecological data that take into consideration the combined effects mentioned above. In this way, predictive microbiology is a powerful tool for food industries because it may help the people in charge to make such decisions.

25.2 Predictive microbiology

25.2.1 History and definition

Food microbiology is a branch of microbial ecology which is aimed at studying the occurrence and growth of microorganisms in foods. Diverse yeast, bacteria and filamentous fungi genera are

associated with food and beverage ecosystems, where they play the double role of both desirable or undesirable (spoilage and pathogen) microorganisms. Drying, fermentation or salting have been used by humans for thousands of years to unconsciously preserve foods free of pathogens, representing an empirical approach to the control of microbial populations. On the contrary, predictive microbiology currently offers a quantitative and objective approach to solve this problem, emerging as a new and crucial element of food microbiology.

Predictive microbiology very probably arose in 1922 with the appearance of the first model that described the thermal inactivation of *C. botulinum* type A spores (Esty and Meyer, 1922). Studies of the potential use of predictive microbiology to describe microbial response can also be found in the 1930s, when Scott (1937) understood the benefit of accumulating kinetic microbial growth data to predict the shelf life and safety of foods.

The origin of 'modern' predictive microbiology can be traced to the 1960s and 1970s, when diverse kinetic and probability models were used to address both food spoilage and poisoning problems (Spencer and Baines, 1964; Nixon, 1971; Genigeorgis, 1981). It was not until the 1980s however, with the development of computer technology and statistical software (which considerably reduced the complexity of calculus for model development) together with the appearance of diverse outbreaks of food poisoning, when predictive microbiology experienced an important expansion. Research articles on predictive microbiology quickly began to appear in the bibliography (Roberts and Jarvis, 1983; Farber, 1986; McMeekin and Olley, 1986; Baird-Parker and Kilsby, 1987). Since then, hundreds of papers, chapter books and general reviews have been written on this topic.

Predictive microbiology is an interdisciplinary area where food microbiologists, statisticians, mathematicians, food technologists and computing scientists all collaborate. In the first book on the subject published by McMeekin *et al.* (1993), predictive microbiology was defined as: 'a quantitative science that enables users to objectively assess the effect of processing, distribution and storage on the microbiological safety and quality of foods'. A later book on the field, published by McKellar and Lu (2003), defines it as: 'the quantitative description of the microbial response in food ecosystems by mathematical models'. Thus, as can be directly deduced from both definitions, a first and important step in the development of a predictive model is the accumulation of data on microbial behaviour in foods. The increasing importance and usefulness of predictive microbiology in foods favoured the publication of a third book on the matter, edited by Brul *et al.* (2007).

A model can be defined as 'the description of a system, theory, or phenomenon that accounts for its known or inferred properties and may be used for further study of its characteristics'. The model is an often simplified description of relationships between observations of the system (dependent variables) and the factors that are believed to cause the observed responses (independent variables). Such a description can be expressed quantitatively in one or more mathematical relationships or equations. A mathematical model can simply describe a collection of data or can represent a hypothesis or series of hypotheses about underlying relationships between the independent variables. The first approach is often referred to as an 'empirical' model, while the latter is describe as 'mechanistic' (McMeekin *et al.*, 2008). Both types of models can be used to assess the risks of food processing and consequently to implement control measures in order to protect the microbiological quality and safety of foods, anticipating the behaviour of pathogen/spoilage microorganisms. It is clear that predictive microbiology has a major role to play in industry and for government and consumers as a modern and essential tool for food management.

25.2.2   Steps to follow in the correct implementation of a predictive model

Models seek to link observations to the variables believed to control them. The successful development of a predictive model in foods and beverages involves a series of steps that include: (1) a detailed

study of the matrix; (2) choice of an appropriate experimental design; (3) data collection; (4) primary modelling; (5) secondary modelling; and (6) model validation. The final result is a safe and useful tool to evaluate the applications of corrective actions during food processing.

25.2.3   Choice of the medium for model development

Beverages and foods are heterogeneous media. They usually provide all the substrates necessary to support microbial growth (sugars, vitamins, nitrogen compounds, oligoelements, etc.) but sometimes also have toxic compounds for microorganisms. Thus, a first and vital step for model development is to obtain detailed physicochemical information on the characteristics of the matrix to establish the levels of the most important compounds present. Industries can manipulate the levels of many preservatives in foods. However, control of the compounds present in the raw material is more difficult because their levels can oscillate depending on many factors.

   Three alternatives exist when choosing the medium for model development. The first option is to use a standard laboratory medium whose components and constants are well known, modified by the factors that the industry can govern (salt, pH, temperature, etc.). The second alternative, and a better approximation to authentic conditions, is to obtain a simulated food ecosystem where many of the food components are added in known concentrations. For example, in wine studies a synthetic must that mimics the natural must composition is widely used (Rossignol *et al.*, 2003). A third and more laborious option is to work directly with the food. In this case, the food has to be previously sterilized (immersion in sodium hypochlorite, UV irradiation, etc.) before inoculation. Inoculation can be carried out with only a single strain of the species or, more appropriately, with a cocktail of strains which leads to a better estimation of the general behaviour of microorganisms (Panagou and Nychas, 2008; Valero *et al.*, 2009).

25.2.4   Experimental design

The experimental design must be chosen as a function of the final objectives, establishing the range and number of environmental factors to study. For instance, if we want to build a polynomial model, the application of central composite or D-optimal designs will be very useful. These designs considerably reduce the total number of experiments to be performed with respect to a factorial design, and consequently save cost and time. On the contrary, a full factorial design will be most appropriate in order to estimate the growth/no-growth boundaries of microorganisms by the use of a logistic model. A third type of design with a huge application in the food industry is the mixture of designs, where the response of microorganisms is associated with the proportion of the components in the mixture. The choice of the most appropriate experimental design is a very important step and will determine the number of experiments to be carried out, the combination of factors and how data will be then analysed and processed.

25.2.5   Data collection

Evolution of microbial populations versus time can be determined in several ways, but the most common procedures are the plate count and optical density (OD) measurements. Data collection is a laborious and expensive step which can be facilitated by the use of automatic apparatus (spiral plate maker, flow cytometers, spectrophotometer, bactometer, etc.). Each method presents its own limitations and advantages. Plate count provides a direct estimation of the total viable cells, but is extremely laborious and time consuming. On the contrary, OD is faster and cheaper, but it is an indirect method that does not offer information on the cell vitality. Other techniques such as flow cytometry have also been successfully used in predictive microbiology to collect growth data (Sørensen and Jakobsen, 1997). An important advantage of this methodology is that cells can be marked individually with different fluorochromes, providing

real-time information of cell vitality (live, death or viable but non-cultivable).

25.2.6   Primary modelling

Microbial population density changes with time in a specified environment can be divided into four phases: lag phase, growth phase, stationary phase and finally death phase. Many primary models have been developed for microbial growth, which include the first three phases mentioned above, but few have been built in the case of inactivation or survival (death phase) as well as for combination of both growth and decline.

Essentially, the function of a primary model is to obtain the growth/inhibition parameters of the microbial population for each of the treatments initially established in the experimental design. In the case of microbial growth, diverse sets of equations (reparameterized Gompertz, Baranyi–Roberts, Logistic, Buchanan, McKellar, etc.) are available to fit the experimental data and obtain the growth parameters of microorganisms (lag phase, maximum specific growth rate and maximum population level). This task is habitually accomplished by curve-fitting with appropriate software using a non-linear regression procedure. Mathematical equations for some of the most widely used primary models can be found elsewhere (McMeekin *et al.*, 1993; McKellar and Lu, 2006; Brul *et al.*, 2007).

While indirect methods such as turbidimetry are widely used in the development of growth models, only viable counts data are valid for inactivation or survival studies. The inactivation of microorganisms may reflect an initial 'shoulder', a linear reduction or sometimes the presence of a tail. Van Boekel (2002) developed an inactivation model based on the Weibull distribution which can be used to determine the shape of the inhibition curve as well as the time necessary to take the first decimal reduction.

Finally, there are other types of primary models which are used to simultaneously fit both the growth and decay phases of microorganisms in foods, such as the quasi-chemical primary model (Taub *et al.*, 2003), the Peleg (1996) model, or the two-term Gompertz equation proposed by Bello and Sánchez-Fuertes (1995). Figure 25.1 shows an example of the three main types of primary models which are habitually used to fit experimental data as a function of time.

A diverse group of software exists to help with this task. DMFIT is an application developed by the Institute of Food Research (IFR), UK which fits bacterial curves where a linear phase is preceded and followed by a stationary phase, calculating parameters such as lag phase, growth rate or maximum population level. Experimental data are directly fitted to diverse primary models, mainly to the Baranyi and Roberts model (1994). A Microsoft® Excel version is available for download (http://www.ifr.ac.uk/safety/DMfit/default.html). GInaFiT (Geeraerd and Van Impe Inactivation Model Fitting Tool) is also a freeware application add-in for Microsoft® Excel aiming at bridging the gap between people developing predictive modelling approaches and end-users in the food industry who are not familiar with or do not have access to advanced non-linear regression analysis tools. The program is able to fit ten different types of microbial survival models on user-specific experimental data relating the evolution of the microbial population with time. It is downloadable via the KULeuven BioTeC homepage (http://cit.kuleuven.be/biotec/downloads.php).

25.2.7   Secondary modelling

Secondary models are built with parameters estimated from primary modelling, and they are used to quantitatively characterize these parameters as a function of the environmental factors included in the experimental design. Sometimes primary model parameters need to be transformed ($\log_{10}$, square root, etc.) before the modelling process in order to homogenize the variance of data and to improve the quality prediction of the secondary model. Once the secondary model has been built, this can be used to predict the response of microorganisms against new combinations of the environmental variables.
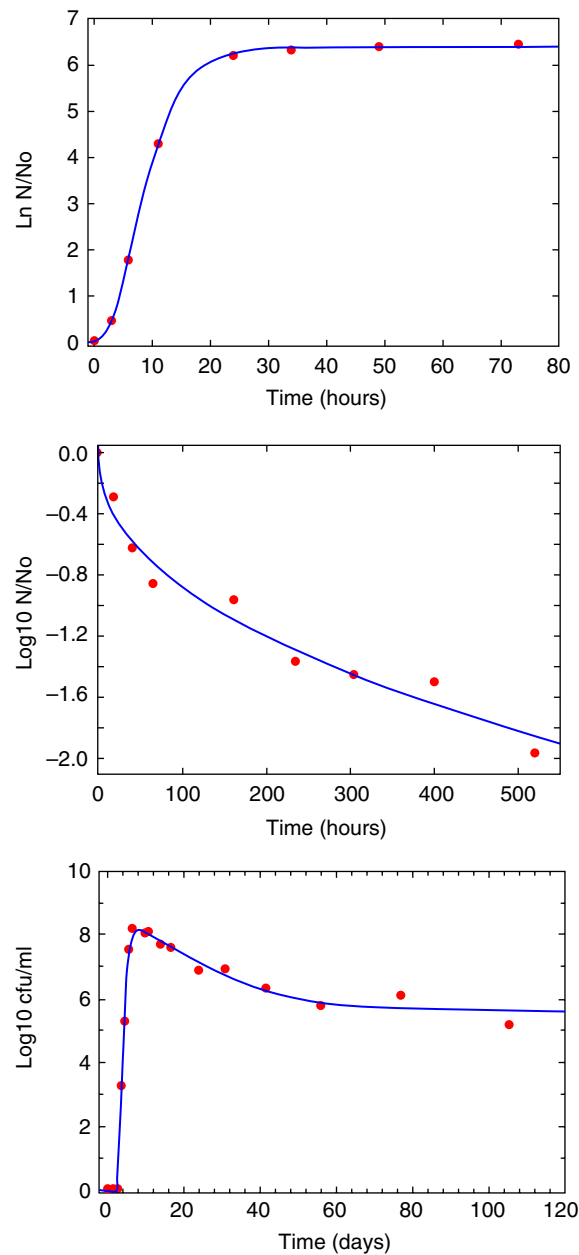
Figure 25.1 Example of diverse primary model fits for the growth (reparameterized Gompertz equation, upper panel), inhibition (Weibull model, middle panel) and growth/decay phases (Bello and Sanchez Fuertes model, lower panel) of microorganisms as a function of time.

The type of secondary model is closely related to the experimental design initially chosen. Secondary models can be easily obtained by non-linear regression or, conversely, be more complex such as polynomial, probabilistic or artificial neural network models that require sophisticated computational software for data processing and analysis. Fortunately, a wide range of powerful statistical software tools is now readily available for use on laptop computers to assist in the development of reliable and robust secondary mathematical models. By means of mathematical analysis of secondary models, the environmental variables with the highest influence on the response can be identified. Some of the most habitual predictive models used in food microbiology are described in Sections 25.2.8–25.2.14.

25.2.8 Square root models

These types of models were initially proposed to describe the effect of temperature on the maximal growth rate, μ_{max}. It has the following expression:

$$\sqrt{\mu_{max}} = b(T - T_{min})$$ (25.1)

where T_{min} is the minimum temperature which supports growth and b is a constant without biological meaning. This equation was extended by Ratkowsky *et al.* (1983) to include the whole biokinetic temperature range. The extended equation is as follows:

$$\sqrt{\mu_{max}} = b(T - T_{min})\{1 - \exp[c(T - T_{max})]\}$$ (25.2)

where b and c are parameters of the model without biological meaning and T_{max} is the temperature above which growth is not possible.

However, in real foods there are more factors that influence microbial growth. For this reason, diverse approaches have been developed to include them in the previous models. Chandler and McMeekin (1989) enlarged the square root model to incorporate the effect of water activity a_w:

$$\sqrt{\mu_{max}} = b(T - T_{min})\sqrt{(a_w - a_{w,min})}$$ (25.3)

where T_{min} and $a_{w,min}$ represents values below which growth is not possible. The combined effect of pH and temperature was also studied by Adams *et al.* (1991) using the following equation:

$$\sqrt{\mu_{max}} = C(T - T_{min})\sqrt{pH - pH_{min}}$$ (25.4)

where pH_{min} has the same meaning as above.

A relevant drawback of these types of models is the presence of some parameters without biological interpretation, as well as the increasing complexity as the number of factors to be studied increases.

25.2.9 Cardinal parameters models

An important step in model development was the introduction of the cardinal parameter models (CPMs) in which the parameters have a biological or graphical interpretation. An obvious advantage of these models is that appropriate starting values for non-linear fitting can be easily found. Their applicability is strongly related to the simultaneous development of the gamma (γ) concept by Zwietering *et al.* (1992), which relies on the concept that many factors that affect microbial growth rate act independently and that the effect of each measurable factor on growth rate can be represented by a discrete term, a fraction of the maximum growth rate (the rate when it is at the optimum level). The overall effect can be represented by the multiplication of all growth-rate-affecting factors. The cumulative effect of many factors at suboptimal levels can then be estimated from the product of the relative inhibition of growth rate due to each factor which is described by a growth factor (γ), a dimensionless measure that has a value between 0 and 1. In this model, the reference growth rate is μ_{max} and the reference levels for temperature (T_{opt}), water activity ($a_{w, opt}$), pH (pH_{opt}), or other factors are those that are the optimum for growth rate. The combined effect of several environmental factors is then determined by the multiplication of their respective γ factors (McKellar and Lu, 2003):

$$\mu_{max} = \mu_{max,opt}\gamma(T)\gamma(a_w)\gamma(pH)$$ (25.5)

25.2.10 Polynomial models

Other well-known types of secondary models for simultaneously studying the combined effect of several factors are the polynomial or response surface (RS) models. The general equation of an RS is:

$$Y = a + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_4 X_1^2 + b_5 X_2^2 + b_6 X_3^2$$
$$+ b_7 X_1 X_2 + b_8 X_1 X_3 + b_9 X_2 X_3 + b_{10} X_1 X_2 X_3 + \varepsilon$$

(25.6)

where Y is the biological parameter to be modelled or its transformations, X_1, X_2 and X_3 are the environmental variables, and a and b_i are the coefficients to be determined by the use of least squares. In case of more independent variables, the model should be properly enlarged.

The structure of an RS model is flexible enough to incorporate even very strong interactive effects by stating the order of the model. For this reason, RS models can provide reasonable predictions of the microbial response in food ecosystems. However, RS models are purely empirical and have certain limitations. A higher-order polynomial model, such as third order with a large number of coefficients, can be expected to show a better fit to primary model parameters but often produces greater topographic complexity. According to the principle of parsimony, a model should contain as few terms as possible. The decision to remove or include a term in the final polynomial equation only depends on whether or not the regression coefficient for the term has a significant effect on the predictive capability of the model.

25.2.11 Probabilistic models

They are used to determine the growth/no-growth boundaries of microorganisms as a function of environmental variables. The general model can be represented:

$$\text{Logit } p = a + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_4 X_1^2 + b_5 X_2^2 + b_6 X_3^2$$
$$+ b_7 X_1 X_2 + b_8 X_1 X_3 + b_9 X_2 X_3 + b_{10} X_1 X_2 X_3$$

(25.7)

where Logit $p = \ln(p/1-p)$. These models incorporate growth/no-growth data (binary response), which are processed by means of a logistic regression model that relate the probability of growth (p) and no-growth ($1-p$) with the environmental factors assayed.

Logistic regression requires a higher number of experiments in comparison with polynomial models, but the great advantage is that they can be easily automatized by means of OD measurements. An important feature of probabilistic models is that the level of probability can be set depending on the level of stringency required, obtaining different growth/no-growth boundaries as a function of the risk that the modeller can assume. Obviously, probabilistic models have a direct application to formula packaging and processing conditions that inhibit the growth of microorganisms (Genigeorgis, 1981; Arroyo-López *et al.*, 2007a, b; Valero *et al.*, 2009).

25.2.12 Neural network (NN) models

A NN model is a computer algorithm which learns or is trained from examples through iteration and automatically derives the mathematical formulae to map the relationships between the input and output data, without any prior knowledge of their relationships. The basic construction of NN consists of input, hidden and output layers, which are composed of neurons that transmit information among the layers. A NN is capable of operating with a large number of neurons at the same time, and for this reason it has been employed in predictive microbiology as an alternative to conventional regression models because of its ability to describe highly complex non-linear problems. Because of the peculiarities mentioned above, these empirical models are also known as 'black boxes'. The advantage of the use of the NN model is derived from its remarkable processing information characteristics, such as: (1) non-linearity; (2) noise intensity; (3) learning and adaptativity; (4) high parallelism; and (5) generalization. An NN model normally has no restriction on the type of relationship between the growth parameters (input patterns)

and the desired outputs. Compared with RS models, the NN model is considered more versatile, flexible and less restrictive because it does not impose any assumptions pertaining to the form of functions. When NN is trained on the appropriate dataset (supervised data learning), it can be used to predict values for unseen cases (generalization) within the experimental region assayed.

25.2.13 Dose response models

There are also models focused on the estimation of the susceptibility and resistance of microorganisms against inhibitory compounds. Lambert and Pearson (2000) developed a simple method for the estimation of the minimum inhibitory concentration (MIC) and non-inhibitory concentration (NIC) of a determined compound using turbidimetry data. The procedure relates the area under the OD/time curves to the degree of inhibition observed, using the ratio of control (absence of inhibitor) to that of the tests (progressive concentrations of inhibitor), referred to as fractional area f_a. As the amount of inhibitor in the well increases, the effect on the growth of the organism also increases. This effect on the growth is expressed by a reduction in the area under the OD/time curve relative to the positive control (optimal conditions) at any specified time. The plot of the f_a versus the decimal logarithm of the inhibitor concentration produces a sigmoid-shaped curve, which can be fit by using a modified Gompertz function for decay (Lambert and Pearson, 2000):

$$f_a = A + C \exp\left\{-\exp\left[B(x - M)\right]\right\} \quad (25.8)$$

where A is the lowest asymptote of f_a (approximately zero), B is a slope parameter, C is the distance between the upper and lower asymptote (approximately 1) and M is the log concentration of the inflexion point.

The whole sigmoid-shaped curve is divided into three regions: zone below the NIC (concentrations of the compound at which no inhibitory effects are observed), concentrations between NIC and MIC (within which growth inhibition progressively occurs) and a third section, above

MIC, where no growth relative to the control is recorded.

25.2.14 Dynamic models

All the secondary models mentioned above are used to estimate the effects of environmental factors at predetermined initial levels. However, it is reasonable to think that dynamic food conditions should also be considered when modelling. In this way, more information has to be incorporated into the existing models, so the physiological response of microorganisms and their microbial dynamic could be explained under varying conditions. The growth process could be modelled by a differential equation, as proposed by McKellar and Lu (2003):

$$\frac{dN_i(t)}{dt} = \mu_i$$
$$\left(N_i(t), <N_j(t)>_{i \neq j}, <\text{env}(t)>, <P(t)>, <\text{phys}(t)>, \ldots\right)N_t$$

$$(25.9)$$

where $i, j = 1, 2\ldots$, is the number of microbial species involved in the process; $N_i(t)$ is the cell density of species i; μ_i is the overall specific rate; $<\text{env}>$ is the physicochemical environmental conditions; $<P>$ is the microbial metabolite concentrations; and $<\text{phys}>$ is the physiological state of the cells. The equation may be enlarged with any other possible influential factor.

25.2.15 Model validation

Regardless of the type of secondary model used, it is necessary to corroborate that the model makes good predictions before it can be used for food safety and quality decisions. Sometimes, the model is built under laboratory conditions so validation in real food is essential to see how the model works in practical situations. A set of new experiments, not included originally in the experimental design, are carried out and the observed responses are compared with those predicted by the models.

The accuracy (A_f) and bias (B_f) factors (Baranyi et al., 1999) have been satisfactorily used in the case of polynomial model validations (Arroyo

et al., 2005; López *et al.*, 2006). The accuracy (A_f) is the sum of absolute differences between predictions and observations and measures the overall model error. On the contrary, bias (B_f) is a multiplicative factor that is used to determine whether the model over- or under-predicts the growth response. Under-prediction of growth (or risk) is dangerous for consumers and an undesirable characteristic of the model. For probabilistic models, validation can be carried out determining the growth/no-growth limits and formulating diverse combinations of the variables where the microorganism is not able to grow (Arroyo-López *et al.*, 2007b). The percentage of correct predictions is then obtained.

In any case, the model is valid only to make predictions in the environmental region where it was built, and cannot be used to extrapolate the response of the microorganisms outside these limits. If the model was built only with a single strain of the microorganism, it would also be convenient to corroborate that the obtained results can be extrapolated for other strains of the same species.

25.3   Microbiological risk assessment

Despite the improvement in food technology and processing, microbiological hazards are associated with some food commodities by their evaluation, control, reduction and/or elimination, an important point to deal with by the different commissions, governments and organisms related to public health. The probability that a microbiological hazard is present in a food constitutes the 'microbiological risk'. The microbiological risk assessment (MRA) is therefore an estimate, through data examination and the use of scientific information, of the risk of food products leading to outbreaks of foodborne illnesses.

These outbreaks are a consequence of a combination of the probability of exposure to the pathogen, the probability of being infected and the severity of the illness. In a complex system such as food production and processing, many factors play a decisive role in microbiological risk; related information is frequently scarce. An effective risk management should therefore be based on an exhaustive examination of all these factors.

MRA is structured in four steps, described in full in the document entitled *Principles and Guidelines for the Conduct of Microbiological Risk Assessment* (Codex Alimentarius, 1999) as follows:

1. *Hazard identification*: 'The identification of biological agents capable of causing adverse health effects and which may be present in a particular food or group of foods'.
2. *Hazard characterization*: 'The qualitative and/or quantitative evaluation of the nature of the adverse health associated with the hazard'.
3. *Exposure assessment*: 'The qualitative and/or quantitative evaluation of the likely intake of a biological agent via food, as well as exposure from other sources if relevant'.
4. *Risk characterization*: 'The process of determining the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment'.

The volume of knowledge necessary to correctly manage a risk assessment requires a cross-disciplinary team (microbiology, epidemiology, medical science, food technology, etc.) who can appropriately handle the available scientific information.

Risk assessment is the basis to the following component in the risk analysis framework, that is, the risk management. Risk management is defined by the FAO/WHO (1997) as 'the process of weighing policy alternatives in the light of the results of risk assessment and, if appropriate, selecting and implementing appropriate control options including regulatory measures'. On this basis, the Codex Alimentarius has developed his own procedure to elaborate Codex Standards (FAO/WHO, 2001).

The last component of risk analysis is risk communication, defined as 'an interactive exchange

of information and opinions concerning risk among risk assessors, risk managers, consumers and other interested parties' (FAO/WHO, 1998).

These three components should be functionally separated to avoid any conflict of interest. Nevertheless, the risk analysis is an interactive process in which the interaction between risk assessor and risk manager, with regard to its practical application, is essential (Figure 25.2) (FAO/WHO, 2006).

On the other hand, the information reported in a MRA can be divided into qualitative and quantitative approaches. From a risk management point of view, quantitative MRA in complex analyses are not shown to provide the best information, since more uncertainty is present in the model parameters. However, they can give more accurate information about risk outputs obtained. Normally, a simple method offers a quick and easy-to-interpret result and a more complex method provides more insight into the identification of the most relevant factors to be considered in risk assessment (Zwietering, 2009). For correct application by risk managers, future trends in MRA should deliver an overview of the determination of target values of the risk factors above which a significant increase in the number of infection cases due to the pathogen is observed, and to the application of potential control measures to reduce illness (Zwietering and Nauta, 2007).

For this purpose, there are currently new risk assessment tools such as the webtool ICRA (Interactive Catalogue for Risk Assessment), which is a 'risk assessment information resource' that provides detailed comparative information on published risk assessments and is designed to be used by risk assessors who are developing a food-chain MRA. On the other hand, iRISK is a web-based tool for 'hazard identification, risk ranking, and prioritization of risks', which can compare and rank risks from multiple food–hazard combinations, calculate public-health outcomes of variations in processing or handling practices and target interventions at all steps in the food supply chain from farm to home.

Despite these advances, an important challenge is to better define one generic risk assessment model to be applied for different countries. This implies a harmonization of data collection methods, methodology, food production systems and international food trade. However, the large variety in practices related to consumer food preparation and consumption complicates a unified approach. A collaborative effort has to be made by all members implicated to obtain a high food safety level.
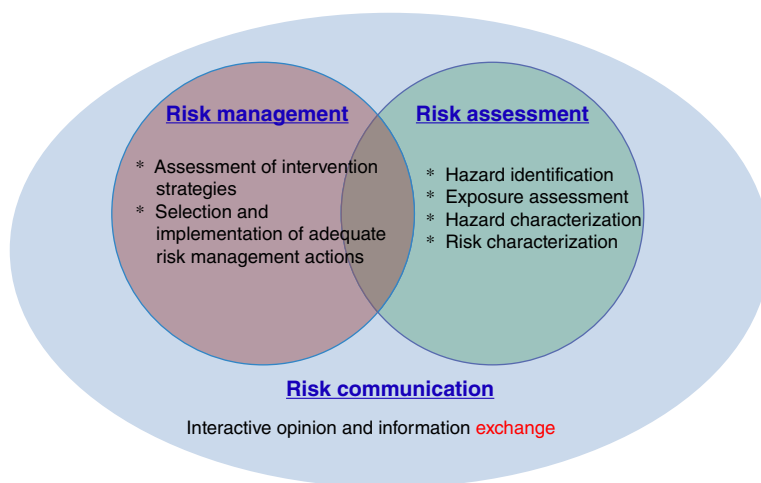
Figure 25.2   Interaction between the different elements of Risk Analyses (FAO/WHO, 2006).

25.4 Software packages and web applications

The routine and successful use of mathematical models by the food industry, governmental or educational agencies will depend on the development of appropriate and useful applications (software packages and online platforms) of easy management. Table 25.1 lists the better-known applications devoted to this purpose on the web, which are usually known as tertiary models. The aim of these applications is to provide automated predictions for diverse pathogens in specific food ecosystems as a function of environmental conditions, which is very useful for microbial risk assessment. The most important applications are as follows.

1. *ComBase*. This is undoubtedly the most ambitious project carried out on a global scale concerning predictive microbiology (www.combase.cc). This initiative emerged from a collaboration between the Food Standards Agency and the IFR, UK, the USDA Agricultural Research, USA and the Food Safety Centre, Australia. ComBase contains thousands of microbial growth and survival curves for both spoilage and pathogen organisms in different food matrices (cheese, vegetables, seafood, etc.), freely available and grouped in numerous microbial models. The main purpose of ComBase is to be a useful and easy tool providing online information about how microorganisms respond to environmental condition changes over the course of time.

2. *Microbial Responses Viewer* (MRV). The MRV is a new database consisting of microbial growth/no-growth data of 19 different microorganisms derived from ComBase. The specific growth rate of each microorganism is modelled as a function of temperature, pH and a_w. The specific growth rate is illustrated using a two-dimensional contour plot with growth/no-growth data. The software allows the user to rapidly view growth/no-growth contour plots superimposed by actual ComBase data.

3. *Pathogen Modelling Program* (PMP). Sponsored by the USDA-ARS, this is a package of models that can be used to predict the growth and inactivation of different foodborne bacteria, primarily pathogens, under diverse environmental conditions in food ecosystems.

4. *Seafood Spoilage and Safety Predictor* (SSSP). The SSSP software was developed by the Danish Technical University and is very useful to predict shelf life and bacteria growth in different fresh and lightly preserved seafood.

5. *Risk Ranger*. Developed by the Australian Food Safety Centre, this is a simple food safety risk calculation tool intended to determine relative risk from different products, pathogen and processing combinations. The program, designed to run in Microsoft® Excel, provides a simple and quick means to develop a first estimate of relative risk.

6. *Risk Assessment Calculator* (RAC). This is another Microsoft® Excel tool which was especially developed for meat products within the EC Fifth Framework Programme (SMAS project). It allows for the estimation of microbial concentration and probability of illness for different pathogens in this type of food.

7. *MicroHibro*. This is an online tool developed by researchers from the University of Cordoba (Spain) especially designed to estimate microbial behaviour of pathogens in specific vegetable and meat products through the application of predictive models. It consists of a user-friendly interface where users can introduce their own models and predictions. Additionally, the tool includes a validation module by which models can be validated with the user's observed data. Finally, the 'Risk Module' allows final concentrations of a given pathogen in a specified process to be estimated by selecting different food chain steps (e.g. growth, transfer and reduction). Users can design specific risk models based on the combination of different basic processes, thus describing changes in concentration and prevalence of a particular microorganism within a specific food chain. In each process, users can define model variables by using point-estimate values or probability distributions. Results can also be analysed

Table 25.1 Most popular tertiary models available on the web to determine the behaviour of pathogens in foods.

Tertiary model	Application/description	Organism	Webpage
Pathogen Modelling Program	To predict the growth and inactivation of different foodborne bacteria under diverse environmental conditions in foods.	USDA-ARS	http://pmp.arserrc.gov/ PMPOnline.aspx
Seafood Spoilage and Safety Predictor	To predict shelf life and bacteria growth in different fresh and lightly preserved seafood.	Danish National of Aquatic Resources	http://sssp.dtuaqua.dk
Risk Ranger	To determining the relative risk from different product, pathogen and processing combinations.	Australian Food Safety Centre	http://www. foodsafetycentre.com. au/ riskranger.php
Risk Assessment Calculator	To estimate microbial concentration and probability of illness of pathogens in foods (mainly meat).	EC Fifth Framework Programme (SMAS project)	http://smas.chemeng. ntua.gr/start.php
MicroHibro	To predict the response and risk of pathogens in meat and vegetable products.	University of Cordoba (Spain)	www.microhibro.com
Sym'previus	Database containing growth, survival and thermal destruction kinetics obtained in foodstuffs for the main pathogen species.	French initiative with public and private partners.	www.symprevius.org
ComBase	To provide online information about how pathogen and spoilage microorganisms respond to environmental condition changes over the course of time.	Food Standards Agency and IFR from UK, USDA and ARS from EEUU and Food Safety Centre from Australia	www.combase.cc
Microbial Responses Viewer	Software which allows the user to rapidly view growth/no growth contour plots of 19 microorganisms in different matrices.	Derived from ComBase	http://mrv.nfri.affrc. go.jp/ Default.aspx#/ Home

by applying scenario analysis and/or sensitivity analysis.

8. *Sym'previus*. This is an extensive French decision support system that includes a database of the response of microorganisms in foods and diverse predictive models for growth and inactivation of pathogenic and spoilage bacteria in foodstuffs. Sym'previus also has diverse online tools (probabilistic module, growth interfaces, growth curve fitting, etc.) which are available by simply taking out a subscription to the operational cell.

In all cases, the guidance of a trained operator is necessary in order to correctly interpret any microbiological significance of data obtained from the output of all these applications.

25.5 Applications and future implications

Mathematical models provide a wide range of possibilities to improve food safety and quality. The availability of quantitative information on the growth and survival of diverse pathogen organisms in food ecosystems will help to make more reliable decisions on the best practices to develop during processing, as well as in the determination and optimization of the appropriate

levels or preservatives (pH, salt, manufacturing practices, etc.) applicable to a specific food. This will considerably reduce outbreaks of food poisoning caused by the production of toxins by foodborne pathogens and, consequently, decrease health expenditures.

There are currently other methodologies that are also being incorporated to achieve more reliable estimations and facilitate predictive model applications. One example is meta-analysis, focused on a systematic analysis of a large collection of data, with the intention of generating standardized and summarized information to produce a global estimate. On the other hand, the emergence of systems biology also affects predictive microbiology, offering new and more mechanistic approaches to yield more reliable and robust predictive models.

By means of mathematical models, food technologists will be able to objectively evaluate the influence of new technological treatments or combinations of preservatives on foodborne pathogens before market launch, obtaining safer, more stable and natural products. Today's consumer demands, among other things, products with lower amounts of calories and sodium levels. The formulation of new products that satisfy these needs, for example adding other lower energetic sugars or salts different from NaCl, must be accompanied by studies that determine their influence on pathogen response.

For all these reasons, predictive microbiology is currently recognized as an important and necessary subdiscipline undergoing expansion within food microbiology, being an important tool for identification of critical control points in the food industry.

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26

Pests in Poultry, Poultry Product-Borne Infection and Future Precautions

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Summary

Consumption of poultry and poultry products is growing rapidly in both developing and developed countries as a result of increasing demand for foods rich in proteins, a world population of over 7 billion, and increased concern about the role of red meat in health and nutrition. This has led to an integration of the poultry industry to achieve enhanced productivity through economies of scale. However, simple mistakes in management in a huge integrated commercial poultry production can have substantial adverse impacts due to a number of pests such as arthropods and rodents associated with foodborne infections. A large number of microorganisms that cause infection in humans or both human and poultry flocks are transmitted to poultry by these pests. However, concerns about pesticide residues are discouraging the use of these agents in poultry industries, thus increasing the reliance on bio-security practices to control infestations. Free range and pasture-flock poultry are popular, but the pasture-flock poultry and poultry products pose potentially higher microbial risks to a large population due to longer production cycle and easy access for pests and other animals. In this chapter, we focus on poultry pests involved in the transmission of microbial pathogens to poultry flocks which result in an increased risk of foodborne infections in humans, loss of food quality, and greater risks for poultry industries.

26.1   Introduction

Food safety can be defined as the system of controls that keeps food and food products free from substances that are hazardous to human health. In this definition, a 'hazard' refers to any physical, chemical, or biological agent or condition that may cause an unacceptable risk to human health (FAO, 1998).

Poultry is a huge industry and poultry animals and corresponding products could be touched and contaminated with foodborne pathogens by soil, water, and also materials within the other poultry products' gut. In the poultry industry, the presence of pathogenic microbes in poultry product is an unavoidable fact of life in both developed and developing countries. Pathogen contamination of poultry is widely known; they infect millions of people every year, resulting in billions of dollars loss due to related health care and production lost. In the United States, the two most prevalent pathogenic bacteria associated with poultry products are *Salmonella* and *Campylobacter* (Bailey, 1993; Wilke *et al.*, 2011). However, within the intestinal lining of poultry such as chicken, many different *Salmonella* and *Campylobacter* serotypes live benignly. Because these pathogens are very resilient, they persist into slaughter and production plants.

For commercial poultry businesses worldwide, pests are a major concern both from the viewpoint of productivity and potential safety concerns. It is well recognized that pests are frequently associated with infrastructural damage (facility damage) or the feeding of contaminated feed and feed ingredients (e.g. mycotoxin-contaminated grains) (Kaufman *et al.*, 2002). Wild pests can be reservoirs and vectors of many agents that cause diseases in food, animals, and human beings (e.g. *Leptospira* spp., *Salmonella* spp., *Campylobacter* spp., *Trichinella* spp., *Toxoplasma* spp.; Hiett *et al.* 2002; Meerburg and Kijlstra, 2007). For instance, high-density confined housing systems widely used in large commercial poultry production operations create environments that favor the survival and development of flies, beetles, and northern fowl mites as a result of manure and poultry litter accumulations. It has been well documented that flies play an important role in transmitting enteric pathogens such as *Salmonella*, enterohemorrhagic *Escherichia coli*, and *Listeria monocytogenes*. Beetles associated with poultry litter and accumulated manure could result in structural damage to poultry housing, serve as reservoirs for potential illness, and create community problems during clean-out of the house (Tomberlin and Drees, 2007). Large populations of northern fowl mite can result in direct economic losses by influencing bird health and production (Meerburg and Kijlstra, 2007; Mullens *et al.*, 2010). Additionally, potential biological control agents such as predaceous mites, parasitoids, and hister beetles that can suppress fly populations are also associated with poultry manure (Rutz and Pitts, 2000).

There are two general strategies for reducing consumers exposure to foodborne pathogens: (1) prevention to stop food hazards from entering the food chain, and (2) intervention to eliminate or reduce pathogens that have entered the food chain to an acceptable level. In current poultry farming practices, a top priority is the development and implementation of effective control measures that reduce zoonotic pathogens, particularly *Salmonella* and *Campylobacter*.

There are many prevalent vectors on poultry farms for both *Salmonella* and *Campylobacter*; however, it is almost impossible to eliminate them from the poultry gut. Fortunately there are many practical measures that can effectively reduce the exposure of these vectors to broiler flocks (Vandeplas *et al.*, 2008; Neal-McKinney *et al.*, 2012). Although people have developed many useful technologies to control these pathogens, due to the complexity of poultry source and point contamination current programs or strategies have not been fully successful in controlling these pathogens. In the past, pest control measures almost totally relied on pesticides for maintaining pest populations below economic injury thresholds or nuisance levels. Since these thresholds were not well defined, control practices were generally not performed until after the pests were found on the poultry or in the poultry house (Blancou *et al.*, 2005).

Small losses due to pests or animal diseases can have significant economic impacts for the farmer and related poultry industries, particularly for large-scale poultry production operations. It is generally agreed that the most effective way to manage these risks is through an integrated pest management approach that is consistent with the current poultry production systems and related management strategies. The objective of this chapter is to describe and discuss important poultry pests, the role of these pests in transmitting microbial pathogens to poultry flocks, and the principles for their management in modern, large-scale commercial poultry production systems.

26.2   The potential risk of contamination in poultry

26.2.1   Conventional poultry

In conventional poultry industry, there are three types of foodborne risk factors affecting the health of humans (Serra *et al.*, 1999; Lupo *et al.*, 2010): (1) microbiological factors such as *Campylobacter* spp., *L. monocytogenes*, and *Salmonella* spp.; (2) chemical factors such as residues from disease-treating medications, feed additives, pesticides, or environmental pollutions; and (3) physical factors such as bone-pieces in meat or iron nails entering from processing.

From a public health viewpoint, the most important group is the first which includes pathogenic microorganisms such as *Salmonella*, *Campylobacter*, *L. monocytogenes*, *Clostridium perfringens*, and pathogenic *Escherichia coli*. Recent concerns about the potential for viruses to change its host ranges is an emerging issue (e.g. avian influenza). On the other hand, helminthes, prions, and protozoa are generally not major sources for zoonotic diseases that are transmitted via poultry products. Microbiological risk factors are so prevailing that they can be found in almost all systems of poultry production. In shell eggs for instance, the most common foodborne microbial pathogens are *Salmonella* spp., *Campylobacter*, *Listeria*, and other enterobacteriaceae. Shell eggs

can be infected via transovarial transmission prior to laying or due to contamination from the environment. Physical damage such as cracks of the egg shell can greatly increase the risk of contamination (Humphrey, 1994; Forshell and Wierup, 2006).

Salmonella enterica is one of the most common pathogens associated with poultry. It is easily transmitted during handling and processing of poultry products, especially non-processed and non-heat-treated products. As poultry producers increase the size of their operations to take advantage of the economies of scale, the number of birds that can be affected by a transmission event increases significantly. This is also true with the increasing internationalization of animal feed providers, live animals, and related avian dietary supplements. Foodborne *Salmonella* infections in human beings are a well-documented public health problem in developed countries. For example, in the United States non-typhoidal *Salmonella* are estimated to be the cause of 19,336 hospitalizations and 378 deaths annually (Scallan *et al.*, 2011). Poultry and egg products are among the foods that are commonly implicated in foodborne *Salmonella* outbreaks.

The potential consequences of *Salmonella* infection can worsen due to emergence of multiple antibiotic-resistant strains, partly resulting from excessive application of antimicrobials in animal feed and treating of those animals (Dione *et al.*, 2012). The virulence of *Salmonella* depends not only on its ability to avoid the host's defenses and invade non-phagocytic cells, but also its resistance to environmental factors (Jones and Falkow, 1996).

During the past decade there has been an increasing appreciation that there are a wide variety of foods that are associated with human salmonellosis, for example fresh produce. However, poultry and poultry products remain a significant contributor to the overall burden of foodborne *Salmonella* infections. One interesting finding was that the incidence of poultry contamination with *Salmonella* is higher in summer (23.6%) than in winter (12.9%), which reveals that designed processing technologies should be

sufficiently effective considering the effects of weather in order to avoid human infection by *Salmonella* (Mahmud *et al.*, 2011). Another contribution of human salmonellosis is due to the appearance of multi-resistant strains because of the usage of disinfectants in the hatcheries. A previous study identified the same resistant *Salmonella* strain from different processing plants (Logue *et al.*, 2003).

Campylobacter is another major foodborne pathogen that is often associated with poultry products. Although the bacterium causes gastro-enteritis in humans, it is part of the normal microbiota of the avian intestinal tract where it resides without any adverse effects (Shane, 1992). *Campylobacter jejuni* and *Campylobacter coli* cause diarrhea, gastro-intestinal pain and nausea in humans (Zheng *et al.*, 2006). Specific genotypes of *C. jejuni* are a leading cause of Guillain–Barré syndrome, relatively rare but highly serious sequelae that damage parts of the human peripheral nervous system. Scientists have actually identified multiple strains in the majority of flocks of broiler chickens (Petersen *et al.*, 2001; Ridley *et al.*, 2011), indicating that at the stage of chicken infection there was no control of the pathogen.

It currently remains unclear how chickens become infected with *Campylobacter* before harvesting. Scientists have identified several potential vehicles that introduce the pathogen into flocks such as feed, water, rodents, flies, horizontal transmission among birds, and hatchery contamination (Zhang *et al.*, 2011). While it has been reported that *Campylobacter* can be isolated from chicken oviducts, it is generally agreed that *Campylobacter* spp. are not vertically transmitted; chicks are born *Campylobacter*-free. During commercial rearing, colonization of chicks is generally evident 1–2 weeks after hatching. Infection cannot be detected until 1–2 weeks after hatching of the young chicks. It was deduced that pathogen contamination was traced back to hatcheries and that facility layers might infect the eggs.

Campylobacter is commonly found in raw poultry meat. This pathogen is very vulnerable

to heat treatment, but if the environment contains a small amount of water with some organic components, the pathogen can then survive for up to several months. Warm-blooded animals are reservoirs for this pathogen (Jones, 2001; Meerburg and Kijlstra, 2007). Considering that this pathogen is vulnerable, many treatments could successfully eradicate the activity of the pathogen in practical production. However, the overall efficacy of these treatments is unclear due to the potential of recontamination, since processing during slaughter and dressing pose significant opportunities for cross-contamination.

Other bacteria such as *Clostridium perfringens*, *C. botulinum*, *L. monocytogenes*, and *E. coli* O157:H7 have also been reported in poultry products (Colles *et al.*, 2008b; Huneau-Salaun *et al.*, 2010), but are less frequently linked to foodborne illnesses. Parasites of poultry and poultry products that can cause human infection are much rarer. In addition to pathogens associated with poultry animals, pathogens associated with human beings including Enterobacteriaceae members are a major food safety concern in handling these animal food products (Kiilholma, 2007).

26.2.2 Pasture poultry

A general public misconception about chicken meat is that free-range or organic chickens are safer than birds reared by conventional production practices. These opinions appear to arise from misinformation related to the effects of large-scale production in high-density enclosed houses, indiscriminate use of antibiotics for the purpose of growth promotion, and the feeding of hormones, again to promote growth. However, studies have recently shown that there was no statistical difference in the prevalence of *Campylobacter* between organic and conventional production chickens. Further, a number of studies suggest a slightly higher incidence of *Salmonella*-positive flocks for organically reared chickens than conventional chicken.

One report indicated that the prevalence of *Campylobacter*-contaminated broiler carcasses was 75.8%, based on results from many locations

among European Union countries (EFSA, 2010). Furthermore, organic chickens have a higher percentage of potential biosecurity concerns considering these chickens are raised outside more than 1/3 of their whole life; this might result in a higher genetic diversity in serotypes of *Campylobacter* and *Salmonella*, and a greater potential of being exposed to a broader range of pathogens associated with external sources of contamination (Humphrey and Jorgensen, 2006; Meerburg and Kijlstra, 2007). Chickens contaminated with *Campylobacter* or *Salmonella* might also transmit the pathogens to wild rodents living on these organic farms, and those infected rodents will contaminate other chickens and continue the contamination cycle (Henzler and Opitz, 1992). It should be noted, however, that if conventional farms do not have solid biosecurity programs then they will have the same problems as organic farms.

In addition, organic farms also provide an ideal location for wild rodents taking refuge. Organic farms generally feed animals living on these farms in the pasture which attracts more rodents than conventional closed-off broiler houses; organic farms therefore do not have as many protection measures as those of conventional broiler houses. The number of rodents in organic farms might also be greater than conventional broiler houses because organic farms provide more roughage and straw in or around the farm, more spacious lands for poultry animals to live, and there is the smaller possibility of rodent poisons as many organic farms either use smaller quantities or none at all (Meerburg and Kijlstra, 2007).

Pasture-flocks or free-range flocks typically have a higher microbial risk for several reasons. First, it is difficult to control the flock's exposure to various pests. Pasture-flocks are raised in an open environment, which means the outside birds have greatly increased exposure to potential vectors. For example, flies and other flying insects inside compared to outside poultry houses are often distinctly different species. Wild birds become a critical issue since they often migrate long distances, thereby breaking down geographical barriers to pathogen dissemination. Further, within the geographical locale of a farm, wild birds are likely to visit a variety of sites including other animal facilities, human waste sites, and other potential sources of contamination. Second, eggs from free-range flocks have a propensity to have a higher bacteriological load on the exterior surface of the shell due to floor-laying or contaminated nesting material (Miao *et al.*, 2005). Third, unlike poultry houses which can be readily cleaned and sanitized between flocks, it is much more difficult to achieve sanitary interventions with open range facilities. Therefore, zoonotic pathogens can be transmitted from one generation of poultry to another of the same range (Colles *et al.*, 2008a; Rivera *et al.*, 2011).

26.3 Major sources of pests in poultry

A primary vehicle leading to the colonization of broiler chickens with *Campylobacter* is house flies that gain access to the broiler houses, possibly through ventilation inlets. House flies acquire the *Campylobacter* by visiting sites of poultry and other livestock feces and subsequently transporting it to the broiler house. This scenario will be more acute in the summer, when housefly populations reach their peak. Interestingly, the frequency of *Campylobacter* contamination coincides with this period (Patrick *et al.*, 2004; Hansson *et al.*, 2007).

Wild rodents can also infect broiler chickens by transmitting *Salmonella* and *Campylobacter*. A USDA study reported that approximately 3.7% of house mice in layer house environments were positive of *Salmonella enterica* serovar Enteritidis (Garber *et al.*, 2003). Further, the number of birds positive for *S.* Enteritidis in broiler houses with mice was nearly four times higher than that in houses without mice (Garber *et al.*, 2003). Wildlife is especially prevalent due to the large amount of available water, food, and shelter. Rodents can also further expand the contamination of pathogens in surrounding environment. One example revealed that mice isolates had approximately four times the number of *Salmonella* as isolates from contaminated

broiler houses (Henzler and Opitz, 1992). Similar to flies, rodents become infected by these pathogens through feces from various sources such as livestock, wild birds, previous chicken flocks, or their fellow members considering they often live in a large group (Garber *et al.*, 2003).

The pests affecting poultry production can be divided into two categories: premise and ectoparasites pests. The premise pests include darkling beetles ('litter beetles'), flies, moths, cockroaches, and rodents (mice and rats), while the ectoparasites include mites, lice, bedbugs, fleas, and soft ticks. The most common ectoparasite for laying hens is the poultry red mite (*Dermanyssus gallinae*), the vector for transmitting *S*. Enteritidis. The mite transmits *Salmonella* as it feeds from one chicken to another, including transmitting *Salmonella* to chicks (Moro *et al.*, 2009).

26.3.1 Premise pests

26.3.1.1 Beetles ('litter beetles')
There are two species of beetles that are associated with poultry products through manure and litter. One is the lesser mealworm (*Alphitobius diaperinus*), also called the darkling beetle, a pest found in stored grain products. This pest distributes almost all over the world and propagates to numerous populations in the litter of broiler breeder- and grow-out houses, and also exists in the accumulated manure in breeder houses under caged layers or the slats (Axtell, 1999). The other is the hide beetle (*Dermestes maculates*), recognized as a pest of hides, furs, and skins. The larvae and adults of both species are commonly associated with poultry manure and litter (Skov *et al.*, 2004). Generally, the hide beetle is less abundant than the darkling beetle in poultry houses.

Both beetles can result in serious damage as the mature larvae evolve into structural materials, apparently seeking a safe pupation site. The darkling beatle is a carrier, transmitter, and reservoir for a couple of poultry disease-related pathogens including acute leukosis or so-called Marek's disease, fowl pox, many pathogenic *E. coli* serotypes, *Salmonella* species, and tapeworms. The major

beetle pest infesting poultry litter and manure is also called the 'lesser mealworm'.

The hide beetle is a beetle with a distinctive warty or bumpy appearance. It can be found worldwide with more than 300 species. The length of hide beetle is 2.5–20.0 mm. The hide beetle is a scavenger and is normally among the last to feed on the remains of dead animals. Both adult and larvae hide beetle eat feathers, fur, and skin of those dead animals; it can therefore transmit foodborne pathogens from those dead animals.

These beetles can become a public nuisance when the manure/bedding from rearing facilities is deposited on nearby fields and the insects subsequently migrate to neighboring residential communities.

26.3.1.2 Rodents
Rodents include rats, mice, squirrels, ground hogs, and other animals that have continuously growing incisors in both the upper and lower jaws. They are not only a nuisance but can spread disease; they therefore need to be controlled. Currently, the Norway rat is the most common rat in poultry farms from reports. This rat lives inside and outside the poultry house in different places such as burrows in the ground, under the foundations, in the litter of breeder houses, under equipment and facilities, or in wood piles and other debris (Mino *et al.*, 2007; Parshad *et al.*, 1987). They need water daily and prefer fresh food, although they can eat most kinds of food. In general, they are nocturnal and come out for food just after sundown.

The house mouse also eats almost any type of food, but normally feeds throughout the day time, feeding the most at sunset and dawn. House mice can live without free water, and they can get free water from the moisture content in their feed (Allymehr *et al.*, 2012).

Both rats and mice can enter a hole that only appears large enough for their head. For mice a 1/4 inch opening is large enough to allow entry. Generally rodents need three basic requirements: food, water, and harborage. The rodent populations cannot grow too much if one or more of the

requirements are not meet. Monitoring the rodent population is critical and the best way is by cage type traps, allowing the number of rodents caught over a certain period, 1 day for instance, to be counted.

26.3.1.3 Wild birds

Wild birds can transmit disease and parasites including Newcastle disease, avian influenza, fowl cholera, chicken mites, and mycoplasma. Feral birds should not mingle with our poultry flocks. The most effective way to control wild birds is to check poultry house air inlets and ensure that open windows are screened with $3/4 \times 3/4$ inch wire meshes. The other measures include cleaning up any feed spills and accumulated water outside the building, cutting grass and weeds to prevent nesting, and searching for possible nests and roosting areas and removing them in time. Some mechanical frightening facilities are available, but the value of such devices are limited (Darre and Rock, 1995). In addition, trapping birds may require permission and is therefore not a good long-term solution (Axtell, 1986; Spackman, 2009).

26.3.1.4 Flies

Poultry manure which has a certain amount of moisture content provides an ideal habitat for the formation of large populations of the house fly, *Musca domestica Linnaeus* (Insecta: Diptera: Muscidae) and closely related species of 'filth flies' (Szalanski *et al.*, 2004). In addition to concerns about flies being a vehicle for the transmission of infectious agents, they also can be a significant nuisance by disturbing workers and affecting nearby community residences and businesses.

The house fly is the common fly pest with the most persistence. It does not bite poultry but can transmit poultry disease. To control house flies, manure management is the most important strategy. In history, it is considered to have contributed to the spreading of the virus leading to the Newcastle disease outbreak in 1970s (Watson *et al.*, 2007). Today, concerns about flies are mainly due to their nuisance characteristics.

Although limited numbers of flies can travel up to several miles from their breeding location, most of them are limited to within several hundred yards from their breeding sites. On the poultry farm, adult flies feed on various materials such as manure, broken eggs, spilled feed, and decaying organic materials from surrounding sources.

Another category of flies are fruit flies. They are often live in places where food has been rotted and fermented. Adult fruit flies are about 1/8 inch long and generally have red eyes. Fruit flies lay their eggs close to the surface of wet organic materials. They are primarily nuisance pests, but can potentially contaminate food with bacteria and other disease-producing microorganisms.

26.3.2 Ectoparasites

26.3.2.1 Mites

The northern fowl mite, *Ornithonyssus sylviarum* (Canestrini and Fanzago, Acari: Macronyssidae), is widely distributed and the most common ectoparasite. In tropical areas, the most common species may be the tropical fowl mite, *O. bursa* (Berlese). However, there are no major differences of the biology, behavior, and control methods between the two species. Fowl mites are common on chickens, turkeys, and all kinds of wild birds; occasionally they may be found on rodents, but they do not reproduce on rodents (Rassette *et al.*, 2011).

Fowl mites are a common problem in caged layers and breeder flocks. This problem is related to the type of housing and the extent of co-habitation of these cages and flocks. For broiler turkeys and those grown outside houses, fowl mites are not critical considering the very short periods these turkeys stay within the house.

Another important type of mite influencing poultry production is the genus *Dermanyssus* (Acari: Dermanyssidae) with *D. gallinae* (De Geer); this mite is referred to as the chicken mite, red mite, or roost mite. The biological characteristics of the chicken mite are significantly different to those of fowl mites. *Dermanyssus* chicken mites are a potential problem in current

poultry production systems because they are easily accessible to birds and have ample places for them to hide within the house, which is helpful for the life cycle of the mite. Chicken mites are often found in broiler breeder houses since the litter, nest boxes, and slats provide a favorable environment. *D. gallinae* is a critical ectoparasitic pest of poultry and it is a potential pathogen vector. It was found that the high prevalence of *D. gallinae* in layer flocks is linked to the presence of *Salmonella* spp. on infested poultry farms (Hamidi *et al.*, 2011).

Avian resistance to an ectoparasitic arthropod and the corresponding costs to the parasite of that host defense for the northern fowl mite have been studied previously (Owen *et al.*, 2009; Birkett *et al.*, 2011; Rassette *et al.*, 2011).

In Europe, *D. gallinae* is the most important ectoparasite of laying hens economically. Due to pesticide resistance and product withdrawal, control of *D. gallinae* has been hampered. Scientists have proposed integrated pest management (IPM), which is often applied in controlling agricultural pests, as a solution. Essential oils such as garlic and thyme might serve as effective *D. gallinae* acaricides and repellents. Other strategies for controlling *D. gallinae* are using predators and fungi and other husbandry techniques such as adjusting temperature and lighting regimes in poultry farms. In general, potential and promising techniques for controlling *D. gallinae* include novel acaricides, vaccines, biological control using natural enemies or entomopathogenic fungi, animal husbandry, and IPM (Mul *et al.*, 2009).

26.3.2.2 Fleas
In poultry houses, fleas (Siphonaptera) are not common creatures; under specific conditions they can however be abundant ectoparasites. In most cases, fleas are commonly present when the poultry houses are used for breeding and after grow out. The most popular species are cat flea, *Ctenocephalides felis* (Bouche), and European chicken flea, *Ceratophyllus gallinae* (Schrank) (Axtell, 1999). One additional species is the human flea, *Pulex irritans* (Linnaeus), which could also be found infesting flocks. Fleas may

enter poultry houses through infested rodents, cats, or wild birds (Axtell, 1999). Bubonic plague is a disease of rodents caused by bacterial *Yersinia pestis*. Humans and other animals can become infected via infected rat fleas. Human beings, for instance, can get the plague from being bitten by rodent fleas that carry bacterial *Y. pestis* (Microbiology, 2013).

26.3.2.3 Ticks
Ticks are associated with old poultry production systems. In modern poultry production, there are not many cases of ticks in poultry products. The two most common species of ticks viewed as poultry pests are fowl ticks (Acari: Argasidae), *Argas persicus* (Oken), and *Argas radiatus* (Raillet) (Medley and Ahrens, 1970). Wild birds are usually the source of infestation. Most infestations occur in breeder houses where the environment is most compatible with ticks.

More evidence has demonstrated that ticks should be considered as a potential and emergent pest and pathogen vector to human beings and animal hosts such as rural poultry (Evans *et al.*, 2000; Malsure and Kolte, 2001).

26.4   Important poultry-related diseases associated with pests

26.4.1   *Salmonella* and *Campylobacter*

In general, wild birds and mammals are recognized as the main reservoir for *Salmonella* and *Campylobacter* in poultry house and poultry products. They are warm-blooded animals and carry both bacteria in their intestinal tracts without any detectable clinical symptoms of disease in most cases (Blaser *et al.*, 1983; Meerburg and Kijlstra, 2007). Many epidemiology reports have demonstrated that infected wild birds or mammals serve as vectors and infect food animals by transmitting the pathogens *Salmonella* and *Campylobacter*. The impact of *Salmonella* spp. in poultry production is of particular importance

because it is closely related to human health, affecting both food safety and poultry production. The behavior of *Salmonella* spp. is a model for elucidating general pathogen persistence in poultry farming or processing environments; however, most of what we know about this pathogen in the poultry production environment comes from indirect evidence (Park *et al.*, 2008).

Numerous projects have studied *Salmonella* in poultry with the ultimate goal of developing effective strategies to minimize *Salmonella* contamination of raw poultry meat. However, although academic, industry and government regulatory agencies have spent decades studying the problem, there has been little if any decrease of in the incidence of human salmonellosis and *Salmonella* is still present in a significant portion of raw poultry (Cox *et al.*, 2011). Table 26.1 lists examples of risk categories and risk factors that are associated with *Salmonella* contamination in poultry. The achievement of a meaningful reduction in the prevalence of zoonotic agents such as *Campylobater* and *Salmonella* requires both intervention-based and prevention-based controls and methods for monitoring the effectiveness of those efforts. Key to such efforts is identifying and understanding the sources and the mechanisms

by which *Salmonella* and *Campylobacter* microorganisms colonize and thrive within poultry and poultry rearing environments. Such efforts are needed to provide an objective means of evaluating the status of poultry farms, and making farmers aware of relevant farm management techniques (Wilke *et al.*, 2011).

Animals, rodents, and pests are recognized as critical sources of *Salmonella* and *Campylobacter* infections and cross-contamination. Rodents are one of the major sources of cross-contamination and infection of *Salmonella*. A previous report demonstrated that 3-week-old chicks were infected by artificially *S.* Enteritidis-infected mice (Davies and Wray, 1995). Spreading of pathogens in the environment can be increased significantly after exposure by rodents: three times more *Salmonella* were isolated from mice than those from the environment contaminated with poultry wastes (Henzler and Opitz, 1992). It has therefore been suggested that rodents continuously reintroduce unstable and invasive phenotypes to the poultry environment (Van de Venter, 2000; Meerburg and Kijlstra, 2007). An increased rate of introduction of *Campylobacter* to broiler houses is also associated with the presence of rats in poultry farms (Kapperud *et al.*, 1993; Liebana *et al.*, 2003). A study reported that 87% of rat

Table 26.1 Comparison of the reported identified main risk categories and factors of *Salmonella* introduction among findings. Adapted from Wilke *et al.* (2011) (NA: not available).

Risk category	Risk factor	Reference			
		Rose *et al.* (1999)	Poppe (2000)	Snow *et al.* (2010)	USDA (2010)
Poultry house management and poultry house state of repair		Important	NA	Important	Moderate
Delivery and collection of birds		Important	NA	NA	Important
Poultry house hygiene		NA	Moderate	Important	Important
	Disinfection foot dips	NA	NA	Important	Moderate
	Disinfection housing equipment	NA	NA	Important	Important
Pest control		Moderate	Important	Important	Moderate
	Rodents	Moderate	Important	Important	Moderate
	Beetles	Moderate	NA	NA	Moderate
Feed management		Important	Important	NA	Important

fecal samples tested positive for *C. jejuni* (Kasrazadeh and Genigeorgis, 1987). Similar phenotypes of *Campylobacter* were isolated in mice intestines as well as in the environmental samples collected from the poultry farm and production sites (Hiett *et al.*, 2002).

Very often *C. jejuni* was transmitted directly from poultry to humans. Due to this transmission, researchers are considering alternative intervention strategies for controlling colonization and cross-contamination of pathogens in poultry production. It is widely accepted that interventions during poultry production potentially provide the greatest opportunity to reduce the risk of foodborne infections. Application of bacteriocin is one of the strategies to prevent *C. jejuni* transmission during animal production. Application of therapeutic bacteriocin can also reduce the colonization of *Campylobacter* in poultry gut from $>10^8$ CFU g^{-1} of cecal materials to below the detection limit or to low levels (Svetoch and Stern, 2010).

Current pre-harvest methods for reducing *Campylobacter* contamination in poultry production are focused on farm biosecurity measures, decontaminating litter, and providing feed with compounds to inhibit *Campylobacter* and treated drinking water, but these are not enough. Some novel strategies, for instance to control *Campylobacter* at pre-harvest levels, are currently under development. These strategies include probiotics administration, vaccination, antibiotics combined with molecules for preventing the emergence of antibiotic resistance, and antimicrobial alternatives such as bacteriophages and bacteriocins (Pasquali *et al.*, 2011).

The Food Safety and Inspection Service (FSIS) is one of the major agencies in the United States responsible for the safety of poultry products. Control of poultry contamination via digestive tract contents is a major focus of regulatory standards. Control of contamination with feces or ingesta is directly linked to improving microbial safety of poultry and poultry products. Improvement of processing technologies, including reprocessing poultry on a production line that can remove feces and ingesta, should be the focus to improve microbiological safety of poultry products (Rasekh *et al.*, 2005).

The FSIS encourages development of innovative technologies to prevent and improve microbial safety for poultry. In the future, the following aspects should be considered to improve the safety of poultry and poultry products: practical evisceration techniques that do not rupture the crop or intestine; application of appropriate processing practices for decreasing cross-contamination risk via digestive tract contents; and development of alternative technologies or methods to evaluate microbial safety (Rasekh *et al.*, 2005).

26.4.2 Coccidiosis of poultry associated with pest

Coccidiosis has been recognized as of one of the major old and chronic causes of poor performance and lost productivity in poultry. The disease is due to protozoan parasites known as *Eimeria*. *Eimeria*'s oocysts are commonly found in the poultry farms and its surrounding environment. It should be noted that these protozoan parasites are strictly pathogenic to poultry, no other animals are affected (Chapman *et al.*, 2010). Coccidiosis affects mainly the intestinal tract of birds. Coccidiosis decreases animal production and entails huge treatment and prevention costs (Peek and Landman, 2011). Due to this disease, many poultry farmers face significant economical difficulties. Currently, most knowledge of coccidiosis has been obtained from chickens.

Monensin is an effective and specific medicine for preventing and curing coccidiosis. Unlike growth-promoting antibiotics, monensin specifically targets the *Eimeria* parasite. Application of monensin in poultry has been widely used for the last 40 years and is recognized as the most effective available compound. Other alternative medications for coccidiosis are under investigation, as it is highly likely that continuous use of monensin will allow the parasite to develop a natural immunity to the compound (Chapman *et al.*, 2010).

26.5 Current practices of pest control in poultry

The size of a pest population in and around poultry houses depends on abiotic and biotic factors. Abiotic factors refer to conditions of the environment. The most important are temperature and the habitat's physico-chemical properties. Biotic factors refer to the effects of living organisms which include natural enemies such as predators, parasites, pathogens, and competition among the species.

In general, three factors including housing type and management, waste management, and flock management are involved in poultry production and shaped the fundamentals of the abiotic and biotic factors. It should be emphasized that these factors are interrelated (Carey *et al.*, 2004).

26.5.1 Housing type and management

Housing type and management are dependent on the type of birds, budget, and the specific preferences of farmers. Appropriate air flow is necessary to dry the manure and litter to reduce fly breeding and ammonia production (Arends and Robertson, 1986; Kathiresan, 2007; Harrington *et al.*, 2011). For open-sided houses, air flow could be significantly improved by cutting grasses and weeds around the houses. That also reduces rodent invasion due to reduced harborage. The houses should be built on graded land to facilitate easy drainage of rainwater. A poor drainage system around the poultry houses causes fly and other pest problems and structural damage of the foundations. Proper housing and management can reduce the production cost and control pest invasion significantly (Arends and Robertson, 1986; Kathiresan, 2007; Harrington *et al.*, 2011).

26.5.2 Waste management

Waste management refers to the strategy of handling manure, litter, and dead birds. Appropriate housing is the best way to dry the manure, and can also help in the removal of manure for spreading on land and to reduce fly invasion. To save energy, a more common disposal method is to flush the manure to a lagoon and recycle the lagoon water for flushing. Proper design and management of a lagoon is crucial in preventing mosquito breeding (Scovill, 1963). Deep lagoons which are free of vegetation at the steep sides of the land could significantly decrease or totally eliminate the mosquito breeding and infiltration to the farm houses. In both breeder and grow-out houses, the litter may contain a mixture of feces, spilled feed, feathers, wood shavings, or other dry materials. Caution is needed regarding excess spilled feed; this is not only economically undesirable, but also creates a favorable environment for beetle production. Although bird mortality is unavoidable, dead birds should not be left in the house or piled just outside the houses. This could promote the insect invasion by promoting pest production such as blow flies. Currently, accepted popular methods of disposal of dead birds are incineration, composting, and burial (Sander *et al.*, 2002; Blake, 2004).

26.5.3 Flock management

Flock management refers to the administration of the general health of the poultry such as feed and water supply methods and their consumption. Too much water consumption by the poultry, and improper nutrition or gastrointestinal diseases, can cause fluid feces characterized as wet manure or litter and facilitate fly invasion and production. Pest problems are often recognized as an indicator of inappropriate housing, waste, and flock management. These management practices strongly affect the abiotic factors such as moisture content, humidity, temperature, and conditions of the manure and waste. These mal-practices also have effects on the biotic factors. The natural pest and parasite populations are influenced significantly by abiotic conditions. In particular, the condition of the manure or litter affects the habitat and survival abilities of parasites, predators, and pathogens that in turn affect populations of pest species (Guerin *et al.*, 2007; Halvorson, 2009).

Several methods can be applied for controlling and eliminating rodent infiltration in the broiler houses. All places that a mouse can enter into the broiler houses should be blocked off. For instance, broiler sidings and doorways should have an appropriate thickness and structure for preventing rodents from entering into the houses. Other measures such as traps and rodenticides can also be applied to eliminate the risk of mice invasion. In an uninhabited house, fumigants could also be considered to eliminate their in-house hiding places (Brown *et al.*, 2002).

Many measures can also be applied for controlling poultry mites (*D. gallinae*). The most critical for preventing contamination by poultry mites is to keep the broiler houses clean. Red poultry mites spend most of their lives on the birds for blood sucking. Currently, the most common measure against red mites is spraying acaricide insecticide in empty broiler houses. However, long-term use of this method could result in an increasing resistance to this poultry mite. Other methods should be considered, including utilizing predatory insects such as spiders, microbial insecticides in the form of exotoxins from other microorganisms, feeding deterrents, and silica toxins (Huber *et al.*, 2011; Lesna *et al.*, 2012).

Natural biological control such as pest predators can be placed within the manure to control mites, beetles, and parasites commonly found in the poultry houses. This biological control is more practical in cage layer operations since predator populations can be increased. This type of biological control results in the suppression of fly production. To save the beneficial insects, it is critical to avoid spraying insecticides directly on manure except occasionally (Lesna *et al.*, 2009).

Parasitic wasps could be placed to add to the naturally occurring wasp populations. The wasp larva originates from the eggs and feeds on the fly pupa, thus reducing the fly population. When farmers choose this method to control flies, they should not spray insecticides in the poultry house. Small predaceous beetles, or hister beetles, are available from most biocontrol producers and can be released in poultry houses for natural control. If sources are clear, predators and competitors can be used to facilitate their co-existing benefits (Prasad and Snyder, 2004).

Additional methods include the use of adult fly traps, which use sex pheromones or food lures to seduce flies into the traps. Zapper traps utilize light to entice flies and then electrocute them. Other light traps may entice flies to glue boards and flies can be trapped when they touch the board to eating or rest.

Chemical control can also be performed through applying baits, contact sprays, residual and bait sprays, or larvicides. By appropriate management of manure and litter, periodical maintenance of drinker lines, and sufficient ventilation, the environment of a poultry house can be unattractive to flies. Understanding the life cycle of various flies and knowing the most effective control strategy for each stage of fly development can allow producers to take useful measures to prevent fly outbreaks (Axtell, 1986).

26.6   Promising pest control strategies

Microbiological risk assessment (MRA) has been recognized as a key strategy of food safety associated with poultry meat products in worldwide management. The methodologies and critical issues such as uncertainty, model complexity, and model validation are important for the study of MRA (Kelly *et al.*, 2003).

Poultry safety is relevant for both pre- and post-harvest levels of production and there are many critical steps associated with safety measures of poultry products. Figure 26.1 describes the steps involved in farm-to-fork exposures and microbial risk management for poultry and poultry products. Multidisciplinary research is required to characterize and improve the sustainability and quality of poultry production (Jez *et al.*, 2011).

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┌─────────────────────┐
│     Production      │
└─────────────────────┘
      P  │  NM
         ▼
┌─────────────────────┐
│  Transportation and │
│     processing      │
└─────────────────────┘
      P  │  NM
         ▼
┌─────────────────────┐
│ Distribution and    │
│      storage        │
└─────────────────────┘
      P  │  NM
         ▼
┌─────────────────────┐         ┌─────────────────────┐
│    Preparation      │         │    Consumption      │
└─────────────────────┘         └─────────────────────┘
      P  │  NM                            │
         ▼                                │
┌─────────────────────┐                   │
│ Exposure estimation │ ◄─────────────────┘
└─────────────────────┘
```
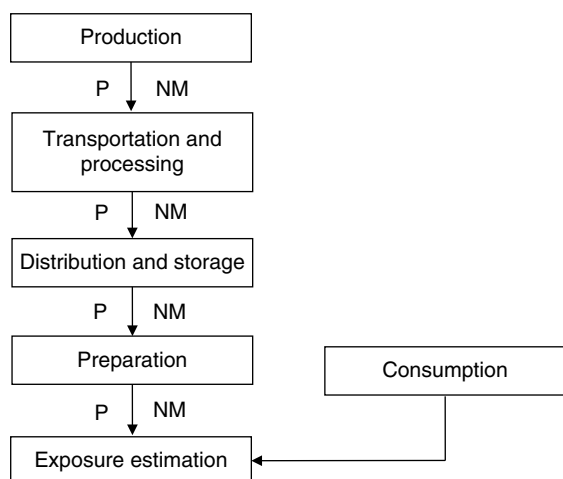
Figure 26.1 Steps involved in farm-to-fork exposure assessment for poultry meat products (P: prevalent; NM: number of microorganisms). Source: Hartnett *et al.*, 2003. Reproduced with permission of WSPA.

26.7 Conclusion and future outlook

Pest control in poultry farms is a prerequisite for biological safety; controlling pests with appropriate chemicals or antimicrobials is essential for chemical safety. Biosecurity is always a primary consideration to prevent colonization and cross-contamination of human pathogens in poultry with potentially disease-related organisms and pests into the facility. Optimal number of flocks, housing, and waste management procedures should be continuously practiced and monitored to assist in controlling pest populations and to prevent invasion by flies, parasites, and other predators. When pest levels are found to be unacceptably high, additional measures should be taken to improve the execution of management practices. The application of insecticide must meet the requirements of the poultry management practices and must not negatively affect the health and environment of flocks in the farm.

Chemicals may sometimes be needed for fly control such as residual spray or insecticide–bait mixtures to lower the adult fly population to a specific acceptable level. However, these chemical applications must be performed with minimal contamination of the manure to maintain fly parasites and predators at the natural populations (Axtell, 1986; Ellis and Scatcherd, 2007).

Many pests on poultry farms can be controlled with proper measures. It is critical to use clean broiler houses to prevent pest invasion and bird contamination. Floors should be cleaned after every flock passes through and properly monitored for the presence of pests. However, it should be noted that the majority of the reported contaminants from pests were not derived through direct contact, but indirectly from contaminated feed and water (Renwick *et al.*, 1992).

Appropriate biosecurity measures should be applied to restrict the amount of contaminant that can spread in poultry farms. Workers should wear appropriate hygiene clothing when dealing with broiler houses (Cardinale *et al.*, 2004). A large portion of *Campylobacter* and *Salmonella* spreading is via the misuse and lack of appropriate clothing and boots and appropriate application of disinfectants in farm practices (Cardinale *et al.*, 2004; Todd *et al.*, 2007). It has been reported that factors contributing to the risks of *Salmonella* contamination in a broiler farm include: neglecting to treat the flock for any diseases; unhygienic conditions in the house; dry conditions under the slats; and frequently walking through the house to pick up dead birds (Volkova *et al.*, 2011).

In addition to temperature control and its role in colonization and transmission of *Campylobacter*, the control of flies is essential, especially during the summer season. A fly screening study revealed that effective control of flies in broiler houses significantly decreased the colonization and contamination of *Campylobacter* compared to the control group (Hald *et al.*, 2007).

Evaluation is the last part of any good integrated pest management (IPM) program. The outcome of the pest control program should be constantly evaluated. This can be performed in several ways by counting pests before and after treatment, rating

comparative damage, comparing costs of pest control periodically, recording pesticide usage, and evaluating its effectiveness. Once pests are under control, it is just a matter of maintaining that level and continuing the monitoring program. Remember that pest problems never can be totally eradicated; they must however be controlled at a manageable level.

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27

Safety of Meat and Meat Products in the Twenty-first Century

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Summary

Product safety concerning meat and meat products has been highly regulated for over a century with an emphasis on government control, visual inspection and, more recently, microbiological testing of end products. Since the 1990s, more systematic approaches to food safety have gradually been applied across the meat industry; hazards, particularly in cooked processed meats and fermented meats, have been controlled adequately. While the hygienic quality of raw meat processing has improved considerably, the expectation that some meat products can be consumed with minimal cooking continues to result in disease. This chapter reviews the history of meat safety with emphasis on raw meat, cooked processed meats and fermented meats, and the application of systematic approaches to providing assurance of safety. It argues against the regulatory imposition of finished product microbiological standards, in favour of demonstrated process control.

27.1   Introduction

Among the wide array of food products available in the market, meat and milk have traditionally been the prototypical products for food safety regulation. In the early twentieth century they were both targeted as products posing risks to human health, and therefore in need of government intervention by way of regulation. Many illnesses attributed to these animal products were zoonotic (e.g. tuberculosis, brucellosis, Q fever) and are now encountered infrequently in food in much of the world. During the twentieth century, the

profile of foodborne diseases and of the foods associated with them changed considerably. For example, the association of familiar pathogens such as *Salmonella* with plant foods e.g. orange juice (Jain *et al.*, 2009) or *Clostridium botulinum* with baked potato (Angulo *et al.*, 1998) and newly recognized foodborne pathogens such as *Listeria monocytogenes* and *Aeromonas* have emerged as foodborne safety concerns. While food safety can no longer be focused only on animal products or on a limited range of pathogens, meat safety remains possibly the most highly regulated sector of the food industry. Much of the regulatory prescription is in the form of carcase-by-carcase inspection and end-product testing directed at a product that is raw and intended to be cooked prior to consumption.

In this chapter we review the origin of meat safety concerns and the regulatory regimes implemented as a result, together with the new paradigms of food safety risk assessment and management arising in the latter part of the twentieth century. Here we present opinion on best practices, cite scientific data in support and provide case studies to provoke consideration of how the available science and technology can be best applied to ensure meat safety. In this chapter we provide examples of these principles in the supply of fresh red meat, ready-to-eat (RTE) cooked meat and fermented meat. Much of the focus of this chapter will be on beef processing with some reference to sheep and, while most of the science cited will come from the Australian context, we believe that the principles illustrated can be applied in all countries and to all slaughter species.

27.2   Where did we start?

In the industrialization of abattoir operations in the late nineteenth century, the safety of workers and of the product was not a high priority. The British medical journal *The Lancet* commissioned a report on sanitary conditions of the world's largest meat operation, Packing Town in Chicago. The reviewer (Smith, 1905) wrote of the construction which allowed '…blood, the splashing of offal and the sputum of tuberculous workers (to) accumulate for weeks, months and years'. Meat was often contaminated with rat faeces and workers ate amid the general filth. The reviewer noted that 'close at hand are closets and they are in some places only a few feet from the food'. In 1905 Upton Sinclair took a commission from the socialist weekly, *The Appeal to Reason*, to investigate the Chicago meat industry. Although under fire from no less a person than the American president, Theodore Roosevelt, the powerful meat industry lobby had effectively stymied a bill to enforce meat inspection standards. Posing as a meat worker, Sinclair infiltrated the Chicago plants where the largely immigrant workforce slaughtered and dressed animals, publishing his findings in *The Jungle* (Sinclair, 1905). Although intended primarily as an exposé of corporate greed and exploitation of workers, the book achieved instant impact in the area of meat safety.

Government regulations, industry lobbies, worker issues and inspection standards under fire may seem as relevant in the twenty-first century as in 1905, but Sinclair changed meat safety forever when he captured the horrendous lack of meat hygiene in *The Jungle*. On reading the exposé, the President, who previously considered Sinclair a 'crackpot' for his socialist views, reportedly threw his breakfast sausages out of the window.

Meat has often been considered a high-risk commodity, which perhaps owes much to *The Jungle* which helped to induce government regulation designed to ensure confidence in the meat supply, and a veterinary profession keen to apply the paradigms of animal diseases and public health. The clear response to the publication of *The Jungle* in the USA was the passing of the Federal Meat Inspection Act (FMIA) 1906 regulating four major areas: livestock (*ante mortem*) inspection, *post mortem* inspection, sanitary standards for meat premises and on-plant inspection and monitoring by the United States Department of Agriculture (USDA).

More than a century later, the FMIA retains the same four basic elements which are replicated

by meat authorities of all countries involved in the global meat trade. However, if meat inspectors from the first half of the twentieth century were to enter a modern abattoir they would find it unrecognizable in terms of hygienic construction, equipment and processing standards; this would also be the case for plants manufacturing ready-to-eat (RTE) meat products. But what effect has the radical improvement in both abattoir and RTE meat operations of recent years had on meat safety?

27.3   Associated risk and public health

At the time the US Act was passed the understanding of food safety was poor. This is reflected in the language used, which is often concerned with preventing 'filthy, putrid, decomposed, unsound, unhealthful, unwholesome, unfit' foods being placed on the market; diseased animals were *ipso facto* unsuitable for human consumption. These concepts are still in place in regulation and within industry practice. The Codex Alimentarius Commission (CAC) *Code of Hygienic Practice for Meat* (2005) requires assessment of meat for safety and suitability for human consumption. Assessment of suitability is often made by regulatory authorities on behalf of consumers, with inspectors judging what a consumer would, or would not, find acceptable. This may not meet changing consumer demands; further, consumers in different countries may have completely different standards. This is putting pressure on industry and governments that are facing population growth and shortages of protein which are predicted to increase to the year 2050 (FAO, 2009).

The latter years of the twentieth century saw the commencement of a significant change in the food safety paradigm: risk was introduced as the defining parameter for managing food safety. This change came as the result of negotiations that formed the World Trade Organisation (WTO) and are embodied in the Sanitary and Phytosanitary (SPS) and Technical Barriers to Trade (TBT) agreements (WTO, 2007a, b). These agreements established the right of countries to protect their citizens from foodborne disease due to imported foods by enacting appropriate requirements at their border. These requirements should be no more stringent than required to provide an appropriate level of protection for that country's consumers.

Furthermore, quantitative risk assessment was prescribed as the approach to be taken in assessing risk. The unit of risk is the likelihood of an adverse effect from the consumption of a meal containing a particular food which, in turn, contains a defined hazard. Quantitative risk assessment (QRA) methods for microbiological hazards in foods have been developed. Risk is a function of both the likelihood of consuming the hazard:food combination (exposure) plus the severity of the adverse event and these are, in turn, a function of consumption patterns, the prevalence/concentration of contamination and the relationship between the dose and response to the hazard. Collecting and analysing sufficient data to comply with the expectations for producing a QRA is no trivial task and risk assessments conducted at the international level frequently take years to complete and typically involve large teams of contributors. Any regulation or import restriction, even those based on risk assessment, can be challenged through the WTO disputes process, and the history of these disputes suggests that it is difficult to produce a risk assessment that cannot be challenged successfully (Orden and Roberts, 2007).

It must also be noted that an importing country's tolerance to risk may differ markedly from that of the exporting country. There is in fact international agreement through the CAC which has produced texts that elaborate on the principles of risk analysis, consisting of three components: risk assessment, risk management and risk communication (WHO/FAO, 2007). Risk management is now considered to be the central component of risk analysis and details how risk managers (for example, national governments): identify foodborne hazards that need to be controlled; call upon risk assessors to provide an understanding of the risk; and make and

implement risk management decisions. These tasks must be performed while communicating the outcomes to stakeholders.

There are a number of other Codex food hygiene texts dealing with basic principles (including HACCP) and with microbiological criteria (CAC, 1997, 2003) that can be applied with or without a risk analysis approach by risk managers. New concepts and metrics have been developed by the CAC to allow risk concepts to be applied to the safety of an end product and the production process.

The concept of a Food Safety Objective has been reviewed in the context of meat safety by Jenson and Sumner (2011). Briefly, a Food Safety Objective (FSO) represents the maximum quantity or prevalence of a microorganism that can be present in a food at the time of consumption and still provide an appropriate level of protection. Based on a risk assessment it is possible to specify Performance Objectives that must be met at some earlier point in the supply chain. It is also possible to define other metrics, such as product temperature or time and temperature of heating product, that are required to prevent microbial growth or to reduce microbes to a safe level. This concept and its application to sampling plans have been explored thoroughly by the International Commission on Microbiological Specifications for Foods (ICMSF, 2002).

The *Code of Hygienic Practice for Meat* (CAC, 2005) provides a risk-based framework for defining meat safety control systems through the supply chain. The principles of the Code dealing with technical issues require meat hygiene control systems to have the primary goal of protecting public health, based on a scientific evaluation of meat-borne risks to health, and using risk analysis principles and HACCP principles for managing hazard throughout the food chain. In this chapter we provide examples of a Codex-consistent approach to risk assessment and risk management, identifying significant food safety controls, possible critical control points and relevant microbiological criteria.

27.4   Meat safety: fresh (chilled and frozen) red meat

Mechanical refrigeration ('cold on demand') revolutionized the food industry, replacing an existing global trade in natural ice. The ability to freeze meat followed and 1879 saw the first vessel fitted with mechanical refrigeration suitable for meat export. After a 60-day voyage from Australia the SS *Strathleven* arrived in London with a 34-tonne cargo of frozen mutton carcases in excellent condition (Farrer, 2005). With their ability to land chilled meat in Europe after a 14-day voyage, South American countries such as Argentina and Paraguay quickly took advantage and landed a product markedly superior to Australian frozen meat. The frozen trade from Australia and New Zealand and chilled trade from South America continued in much the same form until the middle of the twentieth century, apparently without food safety problems, until a Swedish salmonellosis outbreak in 1953 arising from imported veal which had received significant temperature abuse (Lundbeck *et al.*, 1955).

During the 1960s advancement in packaging films and vacuum technology made it possible to reach distant markets with chilled primals and subprimals, with shelf-lives up to 100 days at $-1\,°C$. Around the same time supermarkets installed displays of meat cuts packaged in formats which have developed from the original aerobic overwrapped tray packs to vacuum, gas-flushed and modified atmosphere packs. About the same time fast food restaurants, particularly hamburger restaurants, became popular, spawning a need for frozen manufacturing meat for grinding.

The variety of products, packing formats and cooking styles, in parallel with the development of larger processing units, regionalization and globalization of trade, plus the number of meals eaten out-of-home, have posed new problems in ensuring the safety of meat despite the general improvement in hygienic standards. In particular, as will become apparent, the hugely increased consumption of ground meat in the USA has led to a new global focus on food safety.

27.4.1   Hazards associated with fresh meat

27.4.1.1   Animal health (zoonoses)

Animals coming to slaughter are inspected for a number of conditions which may be detected either at *ante-mortem* and/or at *post-mortem* inspection. Table 27.1 lists the conditions that affect the safety of meat and may be diagnosed by inspection at slaughter. In most developed countries there are very few conditions of animals detectable at *ante-* or *post-mortem* inspection that will affect the safety of the animal for human consumption. An example could be acute enteritis, in which an animal may be excreting large numbers of enteric pathogens which may contaminate meat. Human gastrointestinal anthrax has resulted from the consumption of animals that have died of anthrax and been consumed in the absence of veterinary inspection (Kanafani *et al.*, 2003). The vast majority of conditions are considered to be 'suitability issues' e.g. organoleptic changes in meat such as colour or the presence of parasite cysts (*Onchocerca* nodules).

In other cases, inspection activities may contribute to human health indirectly, such as the diagnosis of tuberculosis (which may be transmitted to humans directly or through the consumption of milk) and leptospirosis (which may be transmitted to man through direct contact with animals and their urine, or with contaminated soil or water in farm environments). Some conditions may be significant to animal health and therefore to the welfare and productivity of the animal, and there is value in providing feedback to farmers with information that will improve husbandry. This is referred to in the CAC meat hygiene code as the 'duality of objectives': concern for both animal and human health. As the health of an animal population improves due to the use of inspection there may come a time where the benefits provided by inspection are outweighed by the act of inspection itself. For example, lymph nodes (including those that appear normal) may harbour pathogenic bacteria since this is part of their role. The act of incision during inspection may spread the bacteria to uncontaminated meat (Arthur *et al.*, 2008). Even inspection without incising lymph nodes may spread contamination over a carcase (Jordan *et al.*, 2012). A number of countries are moving to a more 'hands off' approach when determining the disposition of a carcase. Some countries are actively considering whether major lymph nodes should be discarded as a matter of routine.

27.4.1.2   Chemical hazards

The majority of countries conduct surveys to demonstrate that control over chemical hazards (veterinary and agricultural chemicals, heavy metals and naturally-occurring toxins e.g. mycotoxins) is sufficient to protect human health. Satisfactory levels of control are defined by product samples not exceeding maximum residue limits (MRLs). These are based on chemical risk assessments and generally apply large safety

Table 27.1 Animal diseases that are detectable by veterinary inspection and may be transmitted to man through meat

Cattle	Sheep	Goat	Pig
Acute enteritis (potentially salmonellosis)	Acute enteritis (potentially salmonellosis)	Acute enteritis (potentially salmonellosis)	Acute enteritis (potentially salmonellosis)
Septicaemia, Toxaemia, Pyaemia (potentially salmonellosis)	Septicaemia, Toxaemia, Pyaemia (potentially salmonellosis)	Septicaemia, Toxaemia, Pyaemia (potentially salmonellosis)	Septicaemia, Toxaemia, Pyaemia (potentially salmonellosis)
Cysticercus bovis			*Taenia solium*
Bovine Spongiform Encephalopathy (BSE)			*Trichinella spiralis*
			Spirometra erinacei (sparganosis)

factors to ensure that levels of exposure in humans are well below a no-observed-effect level (NOEL). To achieve MRLs, suitable controls must be applied at the farm when animals are treated with veterinary drugs or chemicals or exposed to agricultural chemicals in feed. In some cases animals need to be withheld from slaughter until compounds are at sufficiently low levels to avoid affecting human health. Considerable effort is made to control access to chemicals and to define suitable doses and periods from exposure to slaughter. Even when MRLs are exceeded, the likelihood of an adverse reaction in consumers is remote. There are few documented cases of chemical residues causing or being linked to adverse health effects. However, Clenbuterol may be fed illegally to pigs to produce leaner meat and its residues have caused stomach pains and heart palpitations in consumers (Anonymous, 2011). Risk assessment to modify a regulatory response was used in 2008 when dioxins from contaminated feed were found in pork in Ireland at up to 100 times the legal limit; potentially, up to 7% of the pork on the Irish market was contaminated. A limited recall was instituted as a precaution, but the risk assessment concluded that the increased burden of dioxin contamination was not a concern (EFSA, 2008).

Trade disputes have arisen from residue concerns. For example, in the mid-1980s the EU banned the use of hormonal growth promotants due to public concerns about adverse health effects. Through an expert panel, the EU concluded that these compounds may be unsafe if consumed at low levels on an ongoing basis. These compounds have been, and are, used in many countries. The USA mounted a successful challenge in the WTO which accepted their proposition that these compounds were safe. Regardless, the EU maintained the ban and the USA were allowed to apply trade sanctions to the EU of an equal value, resulting in the cost of supplying beef to the EU being higher than to other trading blocs. Consumers worldwide increasingly prefer to consume more 'natural' foods and it is likely there will be an increasing focus on chemical residues in the food supply, despite the lack of evidence of adverse public health outcomes. This means that meat producers must be more attuned to these consumer trends and be conscious of how their husbandry practices affect consumer attitudes and perceptions. Systems of transferring the information to meat processors related to treatments that an animal may get during its life, or at least within chemical withholding periods, are being developed in a number of countries to assist with the control of these chemicals.

27.4.1.3   Physical hazards

Some of the physical hazards such as needles, metal, lead shot, injection lesions and other non-food-safety-related pathology are not considered suitable within meat although some, such as needles, may genuinely be considered a safety hazard. Most of these hazards are related to the husbandry of the live animal and can generally have some form of control exerted by the farmer to eliminate or manage the risk (e.g. account for all needles, vaccinate in a defined area on the neck). Some physical hazards are in the control of the meat processor through their maintenance programs in their production facilities; for example, it is now normal practice to install equipment to detect ferrous and non-ferrous objects in meat at the time of packing.

27.4.1.4   Microbiological hazards

Although fresh meat can be contaminated with a large range of pathogenic bacteria (see Table 27.2), the microbiological hazards of most importance, particularly in international trade, are enteric pathogens such as *Salmonella* and Shiga toxigenic (verocytotoxigenic) *Escherichia coli* (STEC or VTEC). Of particular importance in the context of the global meat trade was the 1992–1993 outbreak caused by Enterohaemorrhagic *E. coli* (EHEC), where more than 700 consumers in the NW United States became ill and four children died of Haemolytic Uraemic Syndrome (HUS) following consumption of undercooked hamburgers from a chain of restaurants (Bell *et al.*, 1994). It was not the first time undercooked hamburgers containing STEC had caused illness; Wells *et al.*

Table 27.2   Microbiological hazard risk rating for meat and meat products in Australia (Sumner *et al.*, 2005b). Arbitrary aggregation of Risk Ranger ratings are Low: <25, Medium: 26–40; High >40. Note that a change in risk rating of 6 is equivalent to an order of magnitude change in relative risk as defined in Ross and Sumner (2002)

Product	Identified Hazard	Risk rating	
		Qualitative	Risk Ranger
Red meat entire cuts (steaks, chops, etc)	*Listeria monocytogenes*	Low	Not done
	Staphylococcus aureus	Low	Not done
	Aeromonas	Low	Not done
	Mycobacterium paratuberculosis	Low	Not done
	Bacillus	Low	Not done
	Yersinia enterocolitica	Low	Not done
	Enterohaemorrhagic *E. coli*	Low	Not done
Fresh sausages	*L. monocytogenes*	Low	11 (Low)
Hamburgers	Enterohaemorrhagic *E. coli*	Low	0
Doner Kebabs	*Salmonella*	Medium	40 (Medium)

(1983) reported 'a rare *E. coli* serotype O157:H7' as the cause of food poisoning associated with consumption of hamburgers.

27.4.2   Hygienic processing of meat

Achieving a low microbiological load in fresh meat requires attention to the many unit operations which comprise slaughter, dressing and cutting (fabrication). The gastrointestinal tract and hide are primary sources of contamination and carcase meat must be separated from them with as little cross-contamination as possible; contamination from personnel and equipment must also be minimized. The growth of any microorganisms transferred to the carcase may be controlled by reducing the temperature rapidly and maintaining low temperatures throughout the shelf-life. It is generally considered that the hide is the major source of carcase contamination (Arthur *et al.*, 2010) and bacteria are introduced onto the carcase by the initial hide-incising cuts and during hide removal. Contamination of the hide can be reduced by minimizing stress during transport, by sourcing clean cattle and by managing contamination of hides pre-slaughter. Microbial loadings on the exterior can also be reduced by interventions such as dehairing as is practised in pork and goat processing. Chemical decontamination is also an alternative means of reducing the microbial population of hides of freshly-slaughtered animals (Carlson *et al.*, 2008).

The transfer of microorganisms from the hide to carcase can be minimized by using double knife sanitizing systems (one knife is held in water at a minimum of 82 °C, while the other is being used by the operator), spear cuts, paper to prevent hide roll back, downward hide pullers and trained operators. Contamination of carcases by the contents of the gastrointestinal tract can be minimized by oesophagus tying immediately after bleeding, sealing of the bung during separation of the rectum and reducing rumen volume by ensuring sufficient time off feed prior to slaughter. Fuller accounts of important unit operations in beef and sheep processing are contained in Kiermeier *et al.* (2007) and Kiermeier and Sumner (2009), respectively.

Aerosols during hide removal are also an important potential source of carcase contamination (Schmidt *et al.*, 2012). It is inevitable that some contamination of the carcase will occur during slaughter and dressing, although it can be minimized by operations carried out on the carcases after hide removal and evisceration and by

visual inspection and trimming. Interventions such as steam vacuum and chemical, hot water and steam decontamination can be applied that result in reductions of 1–2 log CFU cm^{-2} (Loretz *et al.*, 2011). Alternative findings on the effectiveness of interventions are presented by Gill and Baker (1988) and Gill and Landers (2003) who did not find significant reductions in *E. coli* populations following steam vacuuming or washing with organic acid. By contrast, thermal pasteurization of carcase sides with hot water around 85 °C or steam was found to result in about 2 log inactivation of *E. coli* (Gill *et al.*, 1999).

Interventions such as those described above and Good Hygienic Practice (GHP) by a well-trained workforce are required. In addition, standards of construction and equipment in meat processing plants are improving, as is separation of hide-on and hide-off areas. The use of gloves to reduce contamination from hands and better use of hand-washing facilities, together with the use of direct and indirect monitoring and supervision systems, all improve carcase hygiene. Contamination from personal equipment and contact surfaces is a significant source of product contamination during cutting and packing (Youssef *et al.*, 2013). When equipment and contact surfaces are properly cleaned, cross-contamination is limited by the quality of the product entering the cutting room, with contact surfaces quickly reaching a microbiological equilibrium with that of the meat passing over them. Poorly cleaned personal equipment and contact surfaces can increase the initial microbiological load on product passing through the cutting room. Growth of microorganisms on contact surfaces and equipment in the cutting room is limited by the temperature of the room and by periodic cleaning. In Australia, cutting rooms operate at temperatures of no warmer than 10 °C, with the aim of limiting growth of enteric pathogens to one generation in a typical 8-hour shift.

Application of active chilling of the carcase (or of cuts, in the case of hot boning) is important to reduce opportunities for the growth of enteric pathogens. Prescriptive rules are often applied such as the need to reduce the carcase surface temperature to below 7 °C (the minimum growth temperature for *Salmonella* and *E. coli*) within a certain time, or the need to reduce deep muscle temperature, which is related to quality rather than safety. Predictive microbiology has been applied to provide an outcomes-based standard that ensures the growth of enteric pathogens is limited, but provides options to the processor on how this is achieved (Ross *et al.*, 2003; McMeekin *et al.*, 2008). The initial stages of chilling are the most significant for control of bacterial growth. While fast initial chilling is desirable, the rate of temperature reduction must be controlled to avoid cold shortening of the muscle fibres leading to poor eating quality. This outcome can be mitigated by electrical stimulation of the carcase to induce rapid onset of rigor, thereby reducing cold shortening during chilling. It is possible to slow the initial rate of carcase chilling and still achieve control over microbial growth because of the effect of surface drying. Distribution of chilled meat in the domestic and export trades is driven as much by shelf-life as by food safety with the former centring on a maximum of 5 °C and the latter as close to 0 °C as possible, ideally near to −1.5 °C, a temperature at which maximum shelf-life is achieved (Gill *et al.*, 1988).

Overall, it can be seen that carcases, meat cuts and meat trimmings can generally be produced with very low populations of the faecal indicator *E. coli*, a situation which can be achieved for both clean (grass-fed) livestock processed at moderate line speeds without interventions and for grain-fed cattle processed at high line speeds with multiple interventions. The common key is that the unit operations involved in freeing and removing the hide, evisceration, chilling and breaking of carcase sides are skill-based.

27.4.3   Risk assessment

Perhaps unsurprisingly, given illnesses and deaths from consuming undercooked hamburgers, a number of risk assessments to estimate the likelihood of illness from consuming hamburgers contaminated with EHEC have been carried out: in Canada (Cassin *et al.*, 1998; Smith *et al.*, 2013); the

USA (Ebel *et al.*, 2004); Ireland (Duffy *et al.*, 2006); Argentina (Signorini and Tarabla, 2009); and the United Kingdom (Kosmider *et al.*, 2011). Equally unsurprisingly, given different conditions of livestock-raising, meat-processing and consumption patterns, estimates of illness vary widely from 1 per 10,000,000 servings (Ebel *et al.*, 2004) to 800 per 10,000,000 servings (Kosmider *et al.*, 2011). While these numbers seem small, given the large number of hamburger servings annually in the USA the burden of disease is significant.

Approaches to these risk assessments were based on through-chain so-called 'farm-to-fork' models, with the exception of the Irish assessment where a survey of retail ground beef was the starting point. Risk profiling is one of the preliminary activities in risk management and has been defined as 'a description of a food safety problem and its context' (CAC, 2003). It involves the systematic collection of information needed to make a decision on what will be done next and where resources should be allocated to provide the detail necessary to undertake a more robust scientific assessment. A risk profile of all raw meat products across the supply continuum for microbial, chemical and physical hazards was undertaken in Australia in 2002–2003. This was performed to enable public health and industry risk managers to prioritize strategic food safety issues for protection of consumers both domestically and in Australia's international red meat markets. To aid in the prioritizing of hazards investigated under the risk profile, risk ratings were generated using a qualitative framework based on ICMSF (2002) and Food Science Australia (2000) and a semi-quantitative spreadsheet tool, Risk Ranger (Ross and Sumner, 2002).

In case of red meat cuts the qualitative estimate of risk of human illness for a range of Gram-negative and Gram-positive pathogens was assessed as low (Table 27.2). An estimate using Risk Ranger was not made where the final cooking step was considered sufficient to inactivate all pathogens. Of meats where the site of microbiological concern was not at the surface (fresh sausages, hamburgers and doner kebabs)

the risk was considered low because (commercially at least) they receive a heat process which is adequate for the destruction of target (Gram-negative) pathogens. However, it was acknowledged that in domestic settings or in commercial establishments where they are deliberately served rare, hamburgers might receive inadequate heat treatment. Using this assumption a risk rating was generated for illness due to EHEC contamination in hamburgers (Table 27.3). Doner kebabs were estimated to be of medium risk, a rating supported by anecdotal linkages between salmonellosis and doner kebab consumption in Australia. The whole-of-industry risk profile was published in three parts: approach and management (Pointon *et al.*, 2006); hazard identification (Sumner *et al.*, 2005a) and risk profiles of hazard:product pairings (Sumner *et al.*, 2005b).

27.4.4   Risk management

27.4.4.1   Microbiological criteria
An indication of how the perception of risk from fresh meat has increased during recent decades may be gained from examining criteria proposed by the ICMSF and the CAC. The 1960s saw the

Table 27.3 Risk Rating summary EHEC contamination in undercooked hamburgers (Sumner *et al.*, 2005b)

1. Hazard severity	Moderate
2. Population susceptibility	General
3. Frequency of consumption	Few times a year
4. Proportion consuming (%)	Everyone
5. Total population	19.7 million
6. Proportion (%) of raw product contaminated (concentration)	0.01% (0.1 g^{-1}, 10 CFU/serve)
7. Effect of processing on hazard	No effect
8. Post processing contamination rate (%)	Nil
9. Post processing control	No increase
10. Increase required to cause infection	100×
11. Effects of preparation before eating on hazard	90% reduction
Predicted cases per annum	6
Risk Rating	36

commencement of work by both bodies seeking *inter alia* to guide and standardize food safety. Initial work by ICMSF sought to standardize methods and sampling for 'lot' acceptance, wherein little (or nothing) was known about the microbiological quality of the product. Microbiological criteria and sampling plans were developed for many foods in international trade (ICMSF, 1974) based on a broad concept of the degree of hazard, which comprised two components: (1) severity of adverse effects, ranging from no effect to serious, life-threatening illness; and (2) whether subsequent processes would increase or decrease the level of hazard.

Five levels of adverse effects and three levels of impact of subsequent processing were defined, creating a series of 15 'cases' with increasingly stringent sampling plans and criteria.

For raw meats, and possibly responding to the Swedish outbreaks of 1953, an early ICMSF sampling plan for *Salmonella* in chilled or frozen carcase meat was assessed as Case 10 where the hazard was adjudged to be serious, incapacitating but not usually life-threatening, and where the food was expected to be consumed cooked. In this case, five × 25 g samples ($n=5$) were required, with no more than one sample ($c=1$) allowed to contain *Salmonella* ($m=0$) but with the aspiration that industry improvement would lead to no sample ($c=0$) containing *Salmonella* (ICMSF, 1974).

The latest edition of the ICMSF sampling plans (ICMSF, 2011) acknowledges that food safety control is no longer accomplished primarily through inspection and end-product testing, but relies on the approach to risk management that has been developed through the work of the CAC. Emphasis is placed on testing of carcases during the process to assess hygienic process control and on testing the processing environment to ensure that cleaning has been adequate. End-product criteria are given for *E. coli* in raw meat (Case 4, $n=5$, $c=3$, $m=10$, $M=100$; where m is the border between acceptable and marginally acceptable counts and M is the border between marginally acceptable and unacceptable counts) and for *E. coli* O157:H7 in beef trimmings used

for ground beef (Case 14, $n=30$, $c=0$, $m=0$), intended for use only in areas of the world where beef is known to be a source of illness.

27.4.4.2 Regulatory requirements

In response to outbreaks involving undercooked hamburgers contaminated with *E. coli* O157:H7, USA authorities imposed the *Pathogen reduction; Hazard Analysis and Critical Control Point (HACCP) systems; final rule* (USDA FSIS, 1996). The rule required that all establishments implement a HACCP plan supported by sanitation standard operating procedures (SSOPs) and good manufacturing practices (GMPs); a zero-tolerance was mandated for visible contamination with faeces and ingesta. Microbiological testing was introduced for both contact surfaces and products (in the latter case, both for indicator organisms and *Salmonella*). Later, the USA declared *E. coli* O157:H7 an adulterant and zero-tolerance was mandated in ground beef and later still in ground beef components. The declaration has resulted in testing of manufacturing beef for the presence of *E. coli* O157 becoming the *de facto* control of this pathogen in the beef supply chain. Thus, by the beginning of the twenty-first century, establishments in the USA and in those countries supplying the USA market were required to produce beef which conformed to criteria for *Salmonella* on carcases and/or *E. coli* O157:H7 in ground beef and its components. This has led to the establishment's testing program becoming a 'disposition CCP' under which a unit (lot) of production cannot be released to the trade until there is confirmation that the pathogen has not been detected in the sampled units.

Together with the adoption of a zero-tolerance policy for *E. coli* O157:H7 was the implementation of testing programs designed to support the concepts of 'adulterant' and 'zero tolerance'. Early sampling plans involved a sample size of 25 g (5 × 5 g samples) per lot of production, later increased to 325 g (5 × 65 g samples), then by so-called N-60 or 'robust' testing involving the collection of 60 surface slices from the external carcass surface. Improvement in analytical techniques has also increased the sensitivity of testing for *E. coli* O157.

This end-product testing program has resulted in pressure being applied to processors to reduce the level of these contaminants to a prevalence at which it isn't routinely detected. Although end-product testing is not considered ideal, this rather blunt regulatory instrument has had the effect of focusing the processor on reducing the risk and avoiding severe regulatory penalties. By contrast, the European Commission has only regulated a microbiological criterion for *Salmonella* in minced meat and meat preparations intended to be eaten raw, using a sample size of 25 g with $n = 5, c = 0, m = 0$ (EC, 2005).

In order for high standards of hygiene to be maintained, the pragmatic approach to setting microbiological criteria for food is to set limits which can be met by the industry operating to the best of its ability. Typically, limits will be set to allow a high proportion of the industry to process without exceeding them, with the intention that the 'worst' performers, say 5–10%, will be stimulated to improve their performance. A problem with setting the microbiological limit too low is that investigations into 'failures' become numerous, possibly without a significant statistical deviation from the mean, and the exercise becomes routine and possibly meaningless. An example of how criteria were set for the Australian meat industry is presented in Vanderlinde *et al.* (2005) in which criteria for *E. coli* and *Salmonella* on chilled beef carcases were set so that 95% of establishments could be expected to conform on a regular basis. Thus, at any one time the stringency of microbiological criteria under which an industry is required to operate will reflect the performance of that sector.

Many countries around the world have introduced microbiological criteria into their regulations or have begun applying microbiological criteria to meat and meat products in international trade. Some of these criteria are focused on wholesomeness (i.e. indicator organisms) while others are focused on pathogens such as *Salmonella*, *E. coli* O157:H7 and specific STEC serotypes. Further, application of these criteria does not always follow the standard risk management framework. Sometimes a product tested at the border is declared unsuitable if any pathogen is detected, even when that pathogen has not been declared an adulterant in the importing country.

An example of a risk-based approach to managing public health is the decision by the Japanese Ministry of Health, Labour and Welfare to require the heating of whole muscle cuts of beef intended for raw meat dishes such as sashimi and yukke (F. Kasuga, pers. comm., 2010). The risk management decision was made following several outbreaks of illness linked to STEC strains in these types of products (Matano *et al.*, 2012). Under the rules, raw meat must be heated to 60 °C at least 1 cm from the surface for at least two minutes, cooled and then the meat must be trimmed 1 cm from each side using special meat-processing equipment. The heating requirement was based on data for the concentration of *E. coli* O157 on beef carcases together with a public health goal to reduce the number of cases occurring each year by 90%. Assumptions were made about a linear correlation between dose and response and cross-contamination, together with a safety factor. A performance criterion of a 4-log reduction for *E. coli* was derived for the heating process. A microbiological criterion for *Enterobacteriaceae* ($n = 25, c = 0, m =$ not detected in 25 g) was derived for process verification using ICMSF approaches.

27.4.5 Performance

There is evidence that microbial loadings have been reduced on carcases and meat cuts. Grau (1979) reported data on beef carcases from a single abattoir that had bacterial counts of close to 4 $\log_{10}$ CFU cm^{-2} in 1937 and 1964, reduced to 2.79 $\log_{10}$ CFU cm^{-2} in 1978. Following the introduction of HACCP, national baseline studies in Australia demonstrated the reduction of average counts on beef carcases to 1.3 $\log_{10}$ CFU cm^{-2} over the years to 2004 (Vanderlinde *et al.*, 1998; Phillips *et al.*, 2001, 2006). Counts of indicator organisms and prevalence of pathogens had fallen to very low levels in boneless beef and sheep meat and on the surface of primals in a national survey conducted in Australia in 2011

(Phillips *et al.*, 2012, 2013). In Canada, the positive effects of skilled knife work by operators on slaughter floors, coupled with thermal processing and organic acid application, can lead to very low populations on the carcase was demonstrated by Yang *et al.* (2012) who described how improved processes and interventions at a large Canadian plant processing up to 280 head/hour led to most carcases having <1 CFU *E. coli* 10,000 cm^{-2} of carcase surface. The researchers then followed carcase sides through the breaking process and found that cuts and trimmings had *E. coli* levels approximately an order of magnitude greater than those on carcases, contamination stemming from conveyors and cotton gloves used by operators (Youssef *et al.*, 2013).

27.5   Meat safety: cooked and ready-to-eat meats

Ready-to-eat (RTE) meats are manufactured from meats and offals using a range of processes including curing, comminuting, cooking and drying. The supermarket of the twenty-first century contains a range of RTE meat products which may be served to order and for consumption over the next few days, as well as a vast number of meats packed under vacuum and modified atmosphere with shelf-lives of many weeks at refrigeration temperatures.

27.5.1   Hazards associated with RTE meats

27.5.1.1   Chemical hazards
To enhance colour and to prevent outgrowth of *Clostridium botulinum* spores, some RTE meats contain nitrite. Nitrite is toxic to consumers at a level above 1500 mg kg^{-1} (ppm) and its addition to the product is a Critical Control Point (for which the critical limit in Australia is 125 mg kg^{-1} at the time of consumption). Pesticide and veterinary drug residues present in the meat used for the production of RTEs will persist in the final product.

27.5.1.2   Physical hazards
Physical hazards are generally the same as those described for fresh meat. Contamination from processing equipment, in particular cutting blades, is a real risk and manufacturers of comminuted products typically have interventions that allow metal contaminants to be detected before the product is released.

27.5.1.3   Microbiological hazards
Earlier, *Staphylococcus aureus* was a common cause of food poisoning due to direct handling by operators coupled with inadequate processing and refrigeration. One of the confounding issues with *S. aureus* contamination is that growth and toxin production during processing may be masked by inactivation of the vegetative cells later in the process. Testing may therefore fail to identify a *S. aureus* problem even when significant amounts of toxin are present in the final product. Where *S. aureus* growth is suspected, testing for toxin rather than the bacterium itself is recommended. The vast majority of RTE meats are cooked prior to distribution, which raises the possibility that *Clostridium perfringens* may replicate if the pathogen is present and if the cooling regime is inadequate. From a risk viewpoint it should be emphasized that *C. perfringens* illness is invariably due to inadequate cooling on non-cured RTE products, particularly in food service settings. According to ICMSF (1996): 'there is no history of *C. perfringens* diarrhea associated with cured meat products since the bacillus is relatively sensitive to sodium chloride and nitrite'.

The microbiological hazard of most concern in RTE meats is *Listeria monocytogenes*. Identified as a pathogen in 1920s as causing listeriosis in animals and occasionally humans, *L. monocytogenes* became a foodborne pathogen of importance in the 1980s. Table 27.4 provides information on selected listerioses associated with a spectrum of RTE meats from a number of countries and illustrates the high proportion of cases which result in death. Numerous outbreaks have been associated with processed meat products including pâté, pork rillettes, hot dogs, corned beef, spreadable sausage and deli meats (ham and

Table 27.4 Selected outbreaks of listeriosis from consumption of ready-to-eat meats (after Sutherland *et al.*, 2003)

Year	Cases (deaths)	Country	Implicated product	Reference
1987–89	9 (2)	UK	Pâté	McLauchlin *et al.* (1991)
1990	11 (6)	Australia	Pâté	Ryser (1999)
1992	279 (85)	France	Pork tongues	Ryser (1999)
1993	38 (11)	France	Pork rillettes	Goulet *et al.* (1998)
1998–99	101 (21)	USA	Hot dogs/deli meats	US CDC (1999)
1999	11 (2)	USA	Pâté	US CDC (1999)
1999–2000	26 (7)	France	Pork tongues	Doronzynski (2000)
2000	39 (1)	NZ	Corned beef	Sim *et al.* (2002)
2002	8 (1)	France	Spreadable sausage	Goulet *et al.* (2002)
2004	(3)	Australia	Corned beef	Givney (2006)
2008	57 (23)	Canada	Deli meats	Weatherill (2009)

emulsified meats, sliced either at the processing plant and vacuum or modified atmosphere-packed, or sliced in the delicatessen).

27.5.2 Processing of RTE meats

Listeria enters the food plant in three ways: in raw materials and ingredients, from the environment and from staff while other pathogens, such as *Salmonella* and *C. perfringens*, are most likely to enter on the raw meat. Once in the processing environment *Listeria* establishes itself in niche environments usually associated with hard-to-clean refrigerated areas. *Listeria* is regularly found in chiller door seals and is a common inhabitant of drains and other non-food-contact areas. Hard-to-clean cutting equipment and conveyor systems are also sometimes contaminated with *Listeria* and are able to contaminate product as it is processed.

27.5.2.1 Cooking
For each cooked product a suitable heat processing step is installed to kill all pathogenic vegetative cells. Delivery of the thermal process is validated by using probes and data loggers and each batch cook is verified to confirm that the scheduled temperature and time have been delivered. Validation is done for each product in each cooker/smokehouse and involves using a time:temperature relationship which provides a '*Listeria monocytogenes* 6D cook' based on

65 °C/10 min at the site of microbiological concern or any other time: temperature regime which provides the same outcome. The thermal process also accounts for other pathogens such as *Salmonella* and the vegetative cells of *C. perfringens*, the latter being more sensitive to heat than *Listeria*.

27.5.2.2 Cooling
Standards set for cooling are geared to systems which traverse the 'danger zone' for *C. perfringens* (*c.* 50–25 °C) with longer times available for RTE cured meats which contain nitrite. The Australian Standard for example requires that, after cooling, the temperature of the meat product at the site of microbiological concern is reduced from 52 °C to 12 °C within 7.5 hours (cured) or 6 hours (uncured products), then reduced to 5 °C within 24 hours of completion of cooking (ANZFRMC, 2007). The performance objective around cooling is to limit any growth of *C. perfringens* to less than 1-log. Similar standards are in place in other countries. Sometimes cooling times for temperatures above 52 °C and below 12 °C are specified, but we are unaware of the microbiological justification because the organism cannot grow above 52 °C or below 12 °C.

27.5.2.3 Preventing Listeria contamination post-cooking
Since cooking processes are designed to eliminate *Listeria* from the product, post-process control of *L. monocytogenes* in RTE meat plants is vital.

However, because slicing/dicing and packing lines become colonized with persistent strains of *L. monocytogenes*, these strains may contaminate final products. *L. monocytogenes* is a tenacious organism, well adapted to surviving and multiplying in food plants. Ghandi and Chikindas (2007) list the organism's survival mechanisms including biofilm formation, quorum sensing and antimicrobial resistance which complement its environmental properties such as ability to grow at low temperatures, low pH and high salt concentrations. For these reasons, many consider *L. monocytogenes* almost impossible to eliminate from food processing plants. Suikho *et al.* (2002) state: 'the occurrence of *L. monocytogenes* in even the most hygienic food processing conditions is difficult to prevent totally' and Chen *et al.* (2003) similarly state: 'data suggests that *L. monocytogenes* cannot be eliminated from the environment or from all food products, and it continues to contaminate RTE products periodically despite the implementation of extensive control measures'.

Earlier, Lundén *et al.* (2003) pointed to the complexity of modern slicing equipment and the difficulty in cleaning and sanitizing it as being responsible for persistent contamination of product with *Listeria*. Of primary importance are the compartmentalization of post-process lines and prevention of any contact with the raw product side of the factory, and the work of Tompkin (2002) and Tompkin *et al.* (1999) is of inestimable value in identifying sites where *L. monocytogenes* can colonize the establishment. Molecular typing of isolates both on final product and in the environment can be valuable in helping to identify critical sources of *Listeria* in the processing environment.

27.5.3 Risk assessment

In the wake of large outbreaks of listeriosis from RTE meats in the USA in 1998 and 1999, a risk assessment of a wide range of food categories was undertaken by the US Food and Drug Administration (FDA) and Department of Agriculture (USDA). The assessment took more than four years to draft, assess and finalize (CFSAN/FSIS, 2003). The assessment estimated cases of listeriosis *per annum* from RTE meats. Of the approximately 1800 cases estimated from all food categories, almost 1600 were attributed to deli meats, with frankfurters eaten without final cooking accounting for a further 30 cases. In contrast with the US risk assessment which found RTE meats to be responsible for around 90% of all listerioses in that country, the Australian assessment attributed around one-third of all listerioses to RTE meats of which almost all were processed meats (Ross *et al.*, 2009a); it inferred that the remaining listerioses in Australia are caused by dairy, seafood and plant-based products, from which there have been documented outbreaks.

27.5.4 Risk management

In Australia, Ross *et al.* (2009b) investigated the effect of several mitigations finding first that retail contamination of cooked meats with *L. monocytogenes* was not of great significance because product sliced at retail usually had a short storage life (no more than seven days); contamination at retail was estimated to cause one additional case of listeriosis every five years. Secondly, reduced prevalence at the processing plant was modelled by assuming enhanced cleaning and sanitation leading to reduced prevalence in product by 90%. Reducing the prevalence in this way was estimated to reduce the number of illnesses by 80%. Thirdly, reduced growth during retail storage was modelled by assuming that lag phase was prolonged by incorporating antimicrobials such as lactate and diacetate into the formulation, which was estimated to reduce the number of illnesses by 86%. The final scenario, reducing levels of *L. monocytogenes* in-pack by orders of magnitude, was modelled by assuming a post-process treatment such as high pressure, high temperature or pasteurizing. The latter intervention was estimated to reduce the number of cases to negligible levels. Manufacturers of these products have adopted various interventions suited to their situation.

27.5.4.1 Breaking the Listeria cycle
It is important to employ processes which break the cycle of *Listeria* colonization and approaches

include raising the temperature of hard-to-clean equipment to around 70°C by enclosing equipment such as slicers and introducing steam, or by moving equipment to cookers. Placing heaters in chillers and bringing their temperature to >50°C for at least 2 hr has also been shown to break the *Listeria* cycle (Eglezos and Dykes, 2011).

27.5.4.2 Preventing growth of Listeria in product

Weak acid salts such as potassium and sodium lactate either singly or in combination with sodium diacetate have been shown to prevent the growth of *L. monocytogenes* on a range of RTE meats. The studies of Samelis *et al.* (2002) on frankfurters, Seman *et al.* (2002) on cured RTE meats and Islam *et al.* (2002) on chicken luncheon meat are typical examples. RTE meat manufacturers are therefore able to formulate products to prevent growth of *L. monocytogenes*, and this task has been facilitated by the development of a calculation tool (Mejlholm and Dalgaard, 2009). The *L. monocytogenes* growth model has been peer-reviewed by international experts (Mejlholm *et al.*, 2010) and predicts growth of *L. monocytogenes* over the required shelf-life of an RTE meat product based on storage temperature, salt content, pH, lactate, nitrite, diacetate and other preservatives.

27.5.4.3 In-pack pasteurization

Pasteurization of a product in its final pack may involve simple technology such as sealing products such as liverwursts or pastes in flexible packs and heating in a conventional water bath for a sufficient time at a temperature to reduce *Listeria* sufficiently. More sophisticated integrated steam pasteurizing and packaging lines are available for high-volume processing, the process delivering a 3-log reduction in *Listeria* for a single layer of frankfurters or a 2-log reduction for interleaved meats (Murphy *et al.*, 2005). High-pressure processing has also been shown to be an effective intervention (Hayman *et al.*, 2004). However, while an option for reducing the initial load of *Listeria* on the product it does not prevent subsequent outgrowth of *Listeria* during prolonged storage. There is anecdotal evidence that products

that are nearly sterile and that have extremely long shelf-lives can allow the occasional growth of *Listeria* to high levels because of the lack of competing microflora.

27.5.4.4 Regulatory requirements

Regulatory approaches to control *L. monocytogenes* in RTE meats vary. In the USA, zero tolerance (non-detection of *L. monocytogenes* in 25 g samples) has been, and remains, the regulatory approach. By contrast, CAC (2007b) makes a significant distinction between RTE foods that support the growth of *L. monocytogenes* and those that do not. The Codex guidelines state that products should contain $<100\,g^{-1}$ *L. monocytogenes* at the time of consumption and that products which do not support the growth of this organism should be subjected to quantitative testing, rather than qualitative testing for presence/absence in 25 g. A number of countries e.g. Germany, Denmark and Canada adopted this approach some years ago. The microbiological criteria specified by Codex are presented in Table 27.5.

27.6 Meat safety: fermented meats

Fermented meats have been made for centuries in many European and Asian countries. European salamis originated in the Mediterranean region and are seasoned with spices, not smoked and usually have Italian or Spanish names. These products spread north to cooler parts of Europe and semi-dry sausages emerged. These are less spiced, smoked at cool temperatures and typically have Germanic names. Asian sausages such as Nham and Musom (Thailand) and Longanisa (Philippines) are fermented at the relatively high ambient temperatures which occur in the region. Fermented meats are made from a range of meat ingredients including pork, beef, mutton and game meats such as kangaroo and venison; fat content is within the range 10–30% and salt, nitrite, starter culture and spices are added to the mix (batter) to provide both safety and flavour.

Table 27.5 Microbiological criteria for *Listeria monocytogenes* in ready-to-eat foods (source: CAC 2007b); *n*: number of samples that must conform to the criterion; *c*: the maximum allowable number of defective sample units in a 2-class plan; *m*: microbiological limit which, in a 2-class plan, separates acceptable lots from unacceptable lots.

Product	Point of application	*n*	*c*	*m*
Ready-to-eat foods in which growth of *L. monocytogenes* will not occur	From the end of manufacture or port of entry (for imported products) to the point of sale	5	0	100 CFU g^{-1}
Ready-to-eat foods in which growth of *L. monocytogenes* can occur	From the end of manufacture or port of entry (for imported products) to the point of sale	5	0	25 g (<0.04 CFU g^{-1})

There are four basic categories of fermented sausage:

1. Moist sausages: these products typically have short fermentation and maturing times and rely on low pH (<4.5) for shelf-stability.
2. Semi-dry sausages: longer maturation periods (depending on diameter) result in a product which is shelf stable when its pH is <5.2 and water activity (a_{w}) is <0.95.
3. Dry, mould-ripened salami: after fermentation salamis are not smoked, but are inoculated with surface mould and ripened at low temperature (10 °C) for up to two months to give an a_{w} <0.90 and pH 5.6–6.0.
4. Very dry, high pH Italian salamis: these sausages are matured for long periods (>2 months) at low temperatures (around 12 °C) and generally have high pH (around 5.8–6.2) and very low a_{w} <0.884.

Fermented products are shelf-stable (able to be stored at room temperature) if a_{w} values are <0.91, or ≤0.95 in combination with a pH of <5.2, or pH <5.0 (Leistner and Rodel, 1976).

27.6.1   Hazards

27.6.1.1   Physical, chemical and microbiological
Physical contamination will be the same as for RTE meats with the possibility of metal contamination from the dicing or mincing equipment. Companies typically have control measures in place to detect metal contamination before the product leaves its control.

Using the wrong nitrite levels represent a similar hazard to that described above for RTE meats. In addition, biogenic amines may be formed during the fermentation process due to the activities of the starter culture.

A number of microbial hazards are recognized (Lücke, 1998), particularly pathogenic *Enterobacteriaceae S. aureus*, *Salmonella* and STEC, all of which have caused outbreaks in fermented sausages. These organisms enter the process through the raw materials and may also colonize the production environment. The conditions in early stages of sausage fermentation (high water activity, high pH and moderate temperature) are conducive to the growth and survival of these pathogens. Numerous cases of salmonellosis have been attributed to fermented sausages, particularly those with high moisture (spreadable sausages). There have been at least three outbreaks of illness from EHEC in fermented sausages. In the USA in 1994, salami was recalled because of *E. coli* O157:H7 contamination (Tilden *et al.*, 1996). In Australia in 1995, *E. coli* O111 in Mettwurst was implicated in around 150 illnesses of which more than 20 progressed to HUS and one child died (Cameron *et al.*, 1995). In Canada, illness was associated with consumption of Genoa salami contaminated with *E. coli* O157:H7 (Williams *et al.*, 2000). *S. aureus* is commonly found in raw meat and if the pH of the fermenting product does not fall to <5.3 (generally within the first 24 hr) or the temperature is not controlled, may grow to a level to cause illness. Guidelines on the time/temperatures during fermentation that control growth of *S. aureus* until the pH has fallen

to <5.3 are provided by the American Meat Institute (AMI, 1982). The organism is not greatly affected by the salt or nitrite in the sausage and may multiply during the early stages of processing when the pH is high and the temperature moderate (Lücke, 1998). Proper use of starter cultures or the addition of gluconodeltalactone (GDL) will prevent the growth of *S. aureus* to levels associated with significant toxin production. While *Listeria monocytogenes* may be found in fermented sausages, the population is usually at a low level and the pathogen is unable to grow under the combination of pH and water activity found in the finished product; it is therefore unlikely to cause disease.

Parasites may be present in raw meat and *Trichinella spiralis* can survive the manufacturing process. Control is via veterinary laboratory inspection of likely sites for the presence of the parasite during pork processing and/or freezing the meat for sufficient time to inactivate the parasite. *Toxoplasma gondii* receives little recognition as a foodborne parasite, but consumption of raw or undercooked meat has been identified as a risk factor in infection. Like other parasites, it seems to be controlled by freezing of the meat or by low water activity in the sausage (Lücke, 1998). A risk-based assessment of various processed meat products (Mie *et al.*, 2008) concluded that fermented products may be at risk if the meat used has not been frozen, if the product has high moisture and if no heat treatment is applied.

27.6.2   Processing of fermented meats

Microbiological safety of the product is dependent on the prevalence and concentration of pathogens in raw materials coupled with the ability of the fermentation and maturing process to control their growth and enhance their inactivation. The concept of the Food Safety Objective may be applied in thinking about fermented sausage production. A certain concentration of a pathogen may be introduced with the raw materials and subsequent processing must result in a concentration of pathogen below the level that will cause illness i.e. provide an acceptable level of protection (ALOP). Testing of raw ingredients and monitoring of processing parameters are therefore important in the control of microbial hazards in fermented meats.

27.6.2.1   Reduction in pH (fermentation)

The increase in pathogen numbers usually occurs in the initial stages of the process. Growth can be controlled by formulating the batter correctly, particularly the concentration of fat, salt and nitrite added to obtain a desirable initial water activity. Rapid pH reduction during fermentation is also required, and is achieved by using a starter culture appropriate for the fermentation temperature and by ensuring that there is sufficient fermentable carbohydrate (sugar) in the batter. GDL can also be added to achieve a rapid pH fall; however, this does not impart the traditional flavours often associated with fermented meat products.

Four groups of starter cultures are used for the manufacture of fermented meats: *Lactobacillus*, *Pediococcus*, *Staphylococcus* and *Micrococcus* (now called *Kocuria*). *Lactobacillus* generally produces lactic acid quickly with few other end-products. *Pediococcus* also produces acid from glucose in addition to other flavour and aroma compounds. *Staphylococcus* and *Micrococcus* produce lactic acid less rapidly than the other starters, but have the benefit of being able to reduce nitrate to nitrite and also enhance flavour and aroma.

Temperature control is important to ensure that the starter culture grows rapidly, utilizes carbohydrate and reduces pH. Traditionally, fermentation was achieved by seeding the batter with fermented meat from a previous batch (termed back-slopping). Unfortunately this method led to inconsistent fermentation, with the possibility that pathogens such as *S. aureus* and pathogenic *E. coli* were able to multiply in the early stages of processing. As a result, and following the outbreaks of the 1990s (see Section 27.6.1.1), many countries mandated the use of starter cultures.

27.6.2.2   Maturing (drying)

Typically, fermented sausages undergo a drying process to a range of a_w values depending on

whether the sausage is moist, semi-dry, dry or very dry (see opening of Section 27.6). Growth of *E. coli* ceases under various combinations of pH and water activity, after which death is almost entirely a function of the temperature at which the product is matured. McQuestin *et al.* (2009) conducted a meta-analysis based on earlier work by Ross and Shadbolt (2001) and produced a model that posits: (1) that *E. coli* will die under conditions in which it cannot grow; and (2) that sausages matured at a reasonably high temperature (>30°C) will achieve a higher level of inactivation of *E. coli* than those matured at a low temperature (<20°C). To produce a safe product the initial level of pathogens must not exceed the level which can be reliably inactivated by the process, the batter must undergo rapid fermentation to prevent their growth and the product must be matured and/or heated sufficiently to inactivate them.

Even in artisanal processes it is important to monitor times, temperatures, pH (or acidity) and water activity (or water loss). In the USA, there is a requirement for fermented sausages to have a process providing for a 5-log inactivation of *E. coli*, which can only be achieved commercially by treating the sausage at a high temperature (about 55°C) for a short time, either immediately after fermentation or after drying. Care must be taken when applying temperature to avoid 'fat-out', the liquefaction and pooling of fat in the sausage casing.

27.6.3 Risk associated with fermented meats

Since the spate of outbreaks reported in the 1990s, control over fermented products appears to have improved. We are not aware of surveys of these products for the presence of pathogens or indicators.

27.6.4 Microbiological criteria

Because testing will not achieve or contribute to the safety of the product, the ICMSF (2011) does not recommend microbiological criteria for fermented products. In Australia, which has no requirement for

processing to achieve a certain inactivation of enteric pathogens such as STEC, fermented sausage manufacturers are required to test for generic *E. coli* in raw meat ingredients and in the product after fermentation and any further processing e.g. heat treatment. Additionally, there are limits for other pathogens that the product is expected to meet, though there is no requirement to test.

27.7 Current status of meat safety and future outlook

The twenty-first century has seen significant investments in the construction and equipping of meat processing plants, together with improved processing and chilling, plus increased testing and regulation. This prompts the question: has the microbiological safety of raw, processed and fermented meats improved as a result? In the case of the latter (RTE and fermented meats) the past decade has seen advances in formulation and in process controls which target enteric pathogens and *L. monocytogenes*. It is now possible for manufacturers of RTE and fermented meat products to control these pathogens both during processing and packaging, as well as through the long shelf-lives demanded by the retail trade. In terms of the safety of raw meat in the twenty-first century, it may be concluded that average microbial populations on meat surfaces in some processing systems have been reduced to a level where there is little benefit to be gained from further reduction. However, the rare presence of pathogens at levels sufficient to cause disease (and outbreaks) when uncooked or undercooked products are consumed points towards an area where further control might be expected of processors. It is these rare high-contamination events that are the likely cause of foodborne illness. Identifying the events that result in these statistical outliers is critical.

While testing of final product lots has been demonstrated to be a very inefficient way of identifying a product which is severely out of specification and likely to cause disease (ICMSF, 1974, 2002) the requirement to test meat continues unabated. However, the ICMSF (2002) and

Codex (CAC, 2007a) discuss the concept of Food Safety Objectives, which is related to achieving an acceptable level of protection and providing a flexible, process-control-driven, through-chain approach to achieving product safety. This approach, if successfully applied to fresh meat, will allow supply chains to provide assurance of safety sought by the authors of meat hygiene regulations over a century ago.

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Application of Hazard Analysis and Critical Control Point Principles for Ochratoxin-A Prevention in Coffee Production Chain

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Summary

Among the wide array of commercial crops marketed internationally, coffee structures itself as an important export commodity fetching substantial foreign exchequer to producers. The presence of mycotoxins (mainly ochratoxin A, OTA) in coffee has been a major barrier of international trade, and greatly affects the economy of the producing country. OTA is a potent carcinogenic, teratogenic, and a nephrotoxic compound, classified as Group 2B (possibly carcinogenic to humans). This toxin is reported to be produced by molds belonging to *Aspergillus ochraceus*, *A. niger*, *A. carbonarius*, and *Penicillium verrucosum*. Coffee beans are prone to mold contamination either during pre-harvest (farm level) or during post-harvest stages (e.g., drying, storage, or transportation). Considering the importance of OTA contamination along the coffee production chain, several works were initiated on their management with India as model producer country. Based on this background, this chapter highlights the possible applications of good agricultural, harvest and management practices (GAP, GHP, GMP) along with the hazard analysis and critical control points (HACCP) approach for the management of OTA-producing molds along the coffee production chain.

28.1   Introduction

Coffee is the most popular and widely preferred non-alcoholic stimulating beverage consumed world over due to its unique taste, aroma and cup quality. Coffee is the second-largest traded commodity in the world market immediately after petroleum. Coffee holds a momentous status in the livelihood of huge populations residing in tropical countries, where it is commonly grown. In general, it has been predicted that nearly 25 million families in 52 exporting countries are dependent upon coffee production. The major coffee-producing countries in the world include: Brazil, Colombia, Ethiopia, Guatemala, India, Vietnam, Indonesia, Mexico, and Uganda.

Coffee is a perennial crop (family Rubiaceae) with only two species cultivated for its popularity: *Coffea arabica* (known as *Arabica*) and *Coffea canephora* (known as *Robusta*). The species *Coffea liberica* (Liberian coffee) is also grown in selected regions of the world and accounts for only 1% of the coffee produced worldwide. Of total global coffee production, the majority (*c*. 70%) is of *Arabica* variety, while the rest is *Robusta* variety.

In India, coffee cultivation is mainly confined to hilly regions along the Western Ghats (Velmourougane *et al.*, 2011a). In India 80% of *Arabica* and 20% of *Robusta* coffee are prepared by the wet method (parchment coffee, where the removal of fruit skin is followed by fermentation, washing and drying), while the remainder is processed by the dry method (cherry coffee, wherein coffee fruits are directly sun dried; Velmourougane *et al.*, 2010b; Velmourougane and Bhat, 2012).

In the present liberalized market scenario, the quality and safety of a food commodity assumes paramount importance among the major export-oriented commodities. The quality of coffee, both visual and organoleptic, is the cumulative contribution of various parameters. The intrinsic quality of coffee is established mainly at the farm level and is dependent on the processing techniques adopted at the farm, which ultimately determines the quality of the beverage (Farah *et al.*, 2006; Velmourougane *et al.*, 2011b).

28.2   Coffee quality and food safety

There are basically two methods of processing coffee which determine the complexity and quality characteristics (see Figure 28.1). These include: (1) the wet method, by which plantation or parchment coffee is produced, and (2) the dry method, by which natural or cherry coffee is produced. Dry processing is mainly used to produce coffee with rich body and aroma and wet processing for fine aroma and acidity (Velmourougane, 2011).

In the present system of coffee marketing, growers tend to sell their produce in the domestic and export markets directly. To meet demand, the quality of coffee needs to be maintained especially when harvest is in surplus and prices are low. In the marketing of coffee beans, the main quality indices of acceptability include raw bean (moisture and water activity levels, size, color, presence of defective beans, foreign matter), roasted bean (with good visual characteristics), and cup (or liquor) quality (body, acidity, and aroma) attributes. Processing is a major activity in the coffee production chain from a quality point of view. Coffee processing encompasses a series of stages, all of which have a distinct purpose.

28.3   Mycotoxins

Mycotoxin contamination in agro-based commodities has been a major and recurring problem in tropical and subtropical regions, where climate (hot and humid) and poor storage conditions are conducive to fungal growth and mycotoxin production. Mycotoxins are toxic secondary metabolites produced by various fungi (molds) (Reddy *et al.*, 2006; Bhat *et al.*, 2010). Of the range of mycotoxins detected, aflatoxins, fumonisins, and ochratoxin A (OTA) are most toxic to mammals (Altuntas *et al.*, 2003). In recent years, the presence of OTA in coffee has been a foremost barrier in international trade, affecting the economy of the producers. OTA (Figure 28.2) is recognized to be a nephrotoxic, carcinogenic, teratogenic, and genotoxic mycotoxin with immune-suppressive
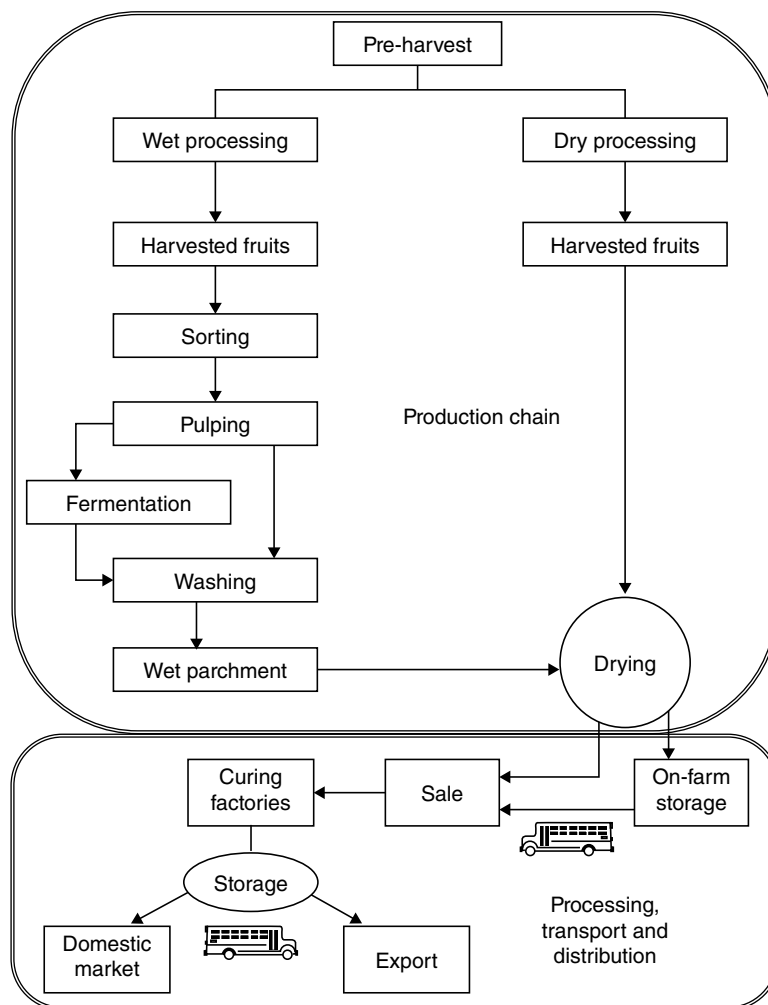
Figure 28.1 Schematic representation of coffee production chain
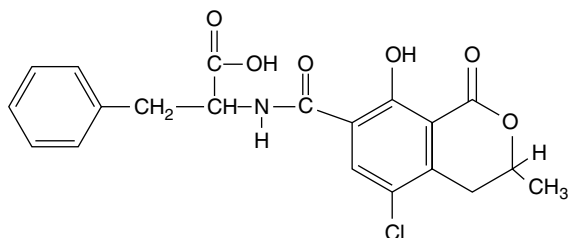
Figure 28.2 Structure of ochratoxin A (OTA)

potential (Bhat *et al.*, 2010; Varga *et al.*, 2010). In addition, OTA is classified as 'possibly carcinogenic' to humans (Group 2B) by the International Agency for Research on Cancer (IARC, 1993). OTA is extensively documented as a global contaminant in green coffee beans and is detected during various levels of processing (Levi *et al.*, 1974; Panneerselvam *et al.*, 2001; Taniwaki *et al.*, 2003; Perrone *et al.*, 2004;

Velmourougane *et al.*, 2005, 2010 a, b; Gopinandhan *et al.*, 2007, 2008a; Batista *et al.*, 2009). The occurrence of OTA in coffee has attracted worldwide attention ever since the Hungarian Health Authorities rejected a major coffee consignment in 1994, citing excess levels of OTA contamination. In view of OTA's toxigenic nature, most coffee-importing countries have implemented maximum permissible limits in coffee products (Taniwaki, 2006; Duarte *et al.*, 2009).

In coffee, molds belonging to *Aspergillus ochraceus*, *A. carbonarius*, *A. niger* (predominant in warm climates), and *Penicillium verrucosum* (predominant in temperate regions) are reported to produce OTA (Moss, 1996; Taniwaki *et al.*, 1999; Joosten *et al.*, 2001; Bayman and Baker, 2006; Pateraki *et al.*, 2007; Hadi and Magan, 2009). However, *A. ochraceus* is reported to be a major source of OTA contamination in green coffee (Frank, 1999).

28.4   Coffee production and OTA contamination

28.4.1   Harvesting

For wet processing, two to three rounds of selective picking of ripened coffee berries are practiced, followed by main harvest and final stripping. Cherry preparation is labor intensive and quite expensive; stripping is therefore followed directly. However, the harvested coffee cherries have to be sorted according to the stages of maturity and with the presence of at least greens and ripes for good quality. In addition, coffee berries should never be allowed to dry on the tree, as this directly supports molds growth leading to inferior quality. Based on working experience, it is always appropriate to use harvest mats to prevent direct fruit contact with the soil and to reduce gleanings (fallen fruits/berries acquired during hand picking, generally collected after completion of fresh harvest). Gleanings are reported to be a potential source of molds as well as mycotoxin contamination, and the contamination rate has been observed to increase

corresponding to the number of days in contact with the soil (Panneerselvam *et al.*, 2000b). Since soil is a potential reservoir of mold propagules, extended contact of the fruit or berries with soil can lead to higher contamination and loss of overall quality. It has been reported that toxigenic molds will displace the initial beneficial yeast population on coffee berry surfaces as the number of days of contact with the soil increases. It has been recommend to collect gleanings as early as possible, and they must be dried and processed as a separate lot (Velmourougane and Bhat, 2009).

28.4.2   Sorting

It is very difficult to pick only the ripe fruits during main harvest; however, harvest lots can be set aside according to different fruit maturity stages after the removal of leaves and twigs. Segregation of fruits prior to pulping promotes effortless and efficient pulping (Figure 28.3). Segregation of coffee cherries based on maturity is reported to enhancing drying and moisture management in dry processing (Panneerselvam *et al.*, 2002; Figure 28.4).

28.4.3   Pulping and fermentation

During coffee processing, delay before processing is unavoidable especially when there is a shortage of drying space. It is advisable to minimize the time lag between harvest and processing (<4 hr), as fruits are susceptible to microbial attack immediately after plucking from the plant. Freshly harvested fruits should always be stored in perforated bags and kept under shade to prevent fruit fermentation within the bags (due to elevated temperatures). Delay in processing has been reported to enhance mold and OTA contamination with cup quality deterioration (Velmourougane *et al.*, 2011b).

Tree-dried cherries usually contain 30–45% moisture with over-ripe berries being highly susceptible to molds attack compared to ripe berries (Panneerselvam *et al.*, 2002). Timely harvesting and processing is therefore of utmost importance to obtain high-quality coffee beans. Generally,

Figure 28.3 Coffee berries during different maturity stages: (a) greens; (b) half-ripes; (c) ripes; (d) over-ripes; (e) tree-dried; and (f) bult lot. After Velmourougane *et al.* (2006a). For color details, see the color plates section.

coffee fruits vary in size resulting in high 'pulper' cuts in beans with more skinned beans. Grading of harvested coffee berries prior to pulping has been reported to minimize pulper cuts. It is therefore advisable to adjust pulper discs and components of pulper to suit coffee varieties (Panneerselvam *et al.*, 2001). One of the most important elements of wet processing is the removal of floats, including those of tree-dried berries in a flotation tank before pulping, which is not achievable in cherry preparation. According to the trials undertaken by Naidu *et al.* (2005) in *Arabica* and *Robusta* coffee, it was found that pre-washing of coffee cherries before pulping or drying can reduce molds on the surface. Similarly, spoiled or rotten (due to mold contamination) cherries float on the surface, while ripe cherries tend to sink to the bottom.

The quality of coffee, both visual and organoleptic, is a collective contribution of different parameters. The intrinsic quality of coffee has been ascertained at farm level and is dependent upon the processing techniques adopted at the farm level (Farah *et al.*, 2006). It has been reported that cautious handling of coffee berries and

appropriate processing techniques can enhance the inherent quality of coffee (Silva *et al.*, 2000). Faulty and unclean processing practices (cleanliness of fermentation tanks, shorter/longer duration of fermentation, water used for processing, etc.) are reported to generate an 'off' taste in the final cup quality (Velmourougane *et al.*, 2006a).

Although there are numerous steps determining the coffee cup quality along the production chain, the fermentation process is considered the most important (Velmourougane, 2012). Demucilization (removal of mucilage, a slippery layer of 0.5–2 mm thickness found around coffee beans) of coffee is performed by several methods such as alkali wash, enzyme treatment, natural fermentation, and direct machine wash (Rothfos, 1985; Avallone *et al.*, 2002). The main objectives of fermentation is to break down the mucilage covering the parchment skin and improve the outer appearance of the raw beans to obtain good liquor quality (Frank *et al.*, 1965).

Based on a wet processing experiment with artificial contamination by *A. ochraceus*, it was observed that the initial load of *A. ochraceus* in fruits is reduced from 74.0% to 11.5% in dried
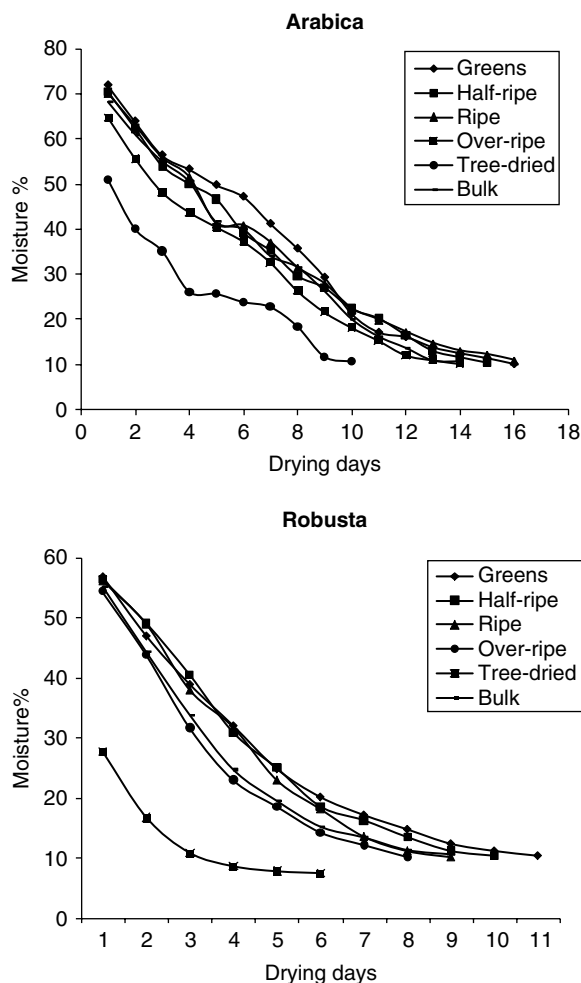
Figure 28.4   Drying characteristics of *Arabica* and *Robusta* coffee based on various stages of maturity .

beans after passing through fermentation, washing and drying (Figure 28.5). The total reduction of *A. ochraceus* was found to be 84.5%. This clearly indicates that even if coffee becomes infected with *A. ochraceous* during initial stages, its load can be reduced via wet processing. Results of the status and survival of inoculated *A. ochraceus* in fruits versus dried beans revealed higher survival rates in freshly harvested fruits (Panneerselvam *et al.*, 2005). Although the incidence of *A. niger* was recorded in both *Arabica* and *Robusta* fermenting mass, the contribution of *A. niger* to OTA production was negligible in

fermented coffee as compared to cherry coffee (Batista *et al.*, 2009).

Further, different types of yeast species have been reported to inhibit molds during coffee fermentation (Grieco *et al.*, 2006; Shetty and Jespersen, 2006; Angioni *et al.*, 2007; Velmourougane *et al.*, 2008a; Bizaj *et al.*, 2009). Specifically, species of genera *Pichia* and *Hanseniaspora* are reported to act as biocontrol agents against *A. ochraceus*, a main contributor of ochratoxin A in coffee (Masoud *et al.*, 2005; Masoud and Kaltoft, 2006). Velmourougane *et al.* (2011a) have shown that inoculation of commercial yeast during the fermentation process could
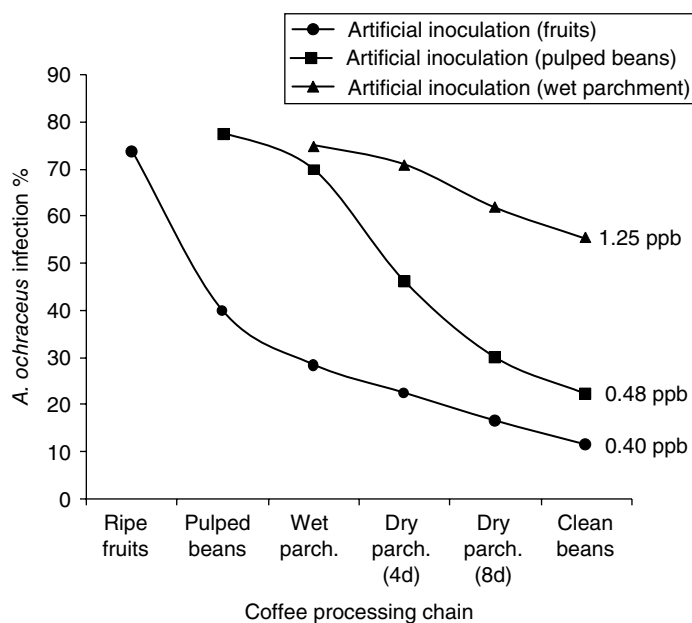
Figure 28.5 Survival of *A. ochraceus* during coffee processing chain (wet processing). After Panneerselvam *et al.* (2005).

considerably reduce the toxigenic mold incidence and OTA formation in green coffee beans.

28.4.4 Drying

Drying of coffee beans is an important technique that determines its shelf-life. Appropriate drying at adequate moisture and water activity levels is important to prevent the colonization of storage molds. Different drying methods have been followed for early drying of the processed coffee beans (Velmourougane *et al.*, 2010b). A suitable drying yard is a pre-requisite for producing high-quality coffee beans. The drying yard must be located at an elevated position at the farm, where maximum sunshine is expected with minimal risk of flooding (via rain) or cross-contamination by animals.

28.4.4.1 Drying surfaces
A good drying yard is also required to prevent contamination and it is recommended that coffee beans be dried on a floor covering of concrete, tiles, brick, granite or any other hard surface. The processed coffee beans should never be dried on uncovered soil or on soil smeared with cow dung slurry, as these surfaces tend to

produce inferior-quality coffee and increase the risk of microbial contamination. It is recommended that coffee plantations lacking an appropriate drying yard facility can dry coffee on plastic or tarpaulin sheets (picking mats), provided the coffee is turned regularly to evaporate condensed moisture on the bottom layer. In addition, the drying yard should be smooth with no cracks in which microbial propagules could harbor and flourish (Velmourougane *et al.*, 2006a).

28.4.4.2 Drying thickness, stirring frequencies and moisture protection
Once the coffee is washed, tray drying (surface drying in sunlight) is recommended to remove excess moisture from the surface of coffee beans. In one of our experiments, we observed that the drying of coffee in a tray ($2\,m \times 1\,m \times 5\,cm$) accelerates the drying process by removing excess moisture accompanied with a reduction of splitting of parchment. Tray-dried coffee beans were found to attract less mold contaminants compared to directly dried beans. The time required for drying parchment and cherries varies according to the drying yard, thickness of the layer, number of stirring days, initial moisture content

(a)

(b)
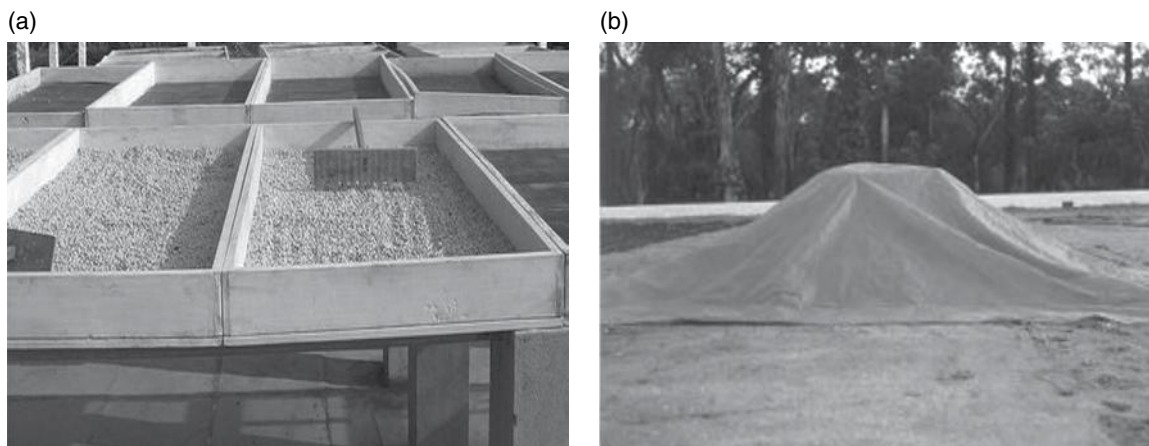
Figure 28.6 Drying techniques to prevent OTA mold contamination in coffee: (a) tray drying; (b) covering during night. Source: Coffee drying yard, CCRI, Chikmagalur, Karnataka

of the beans, sunshine, temperature, and relative humidity. Maintenance of the correct thickness of the layer leads to faster and uniform drying and avoids splitting of parchment and casing of cherry without any mold contamination (Figure 28.6).

Coffee beans should be raked or stirred 4–6 times a day for enhanced removal of water content/moisture to prevent mold growth (Velmourougane *et al.*, 2010b). Raking devices used should never be too hard as they may split the parchment cover and should be provided with rubber beadings. Care also needs to be taken such that fresh cherries are not mixed with partially dried cherries. It has been reported that beans dried above 5 cm thickness in parchment and above 8 cm in cherry lead to a lower cup quality, while longer drying times allow for higher fungal and mycotoxin contamination (Naidu *et al.*, 2005).

During preparation of parchment, cherry coffee is not covered. Parchment coffee with high moisture levels should not be covered until it reaches the desired level of moisture (*c.* 35%); after this moisture level has been reached it requires daily covering. Before covering, coffee lots must be heaped and allowed to attain equilibrium. If coffee lots are covered during hot weather, sweating of beans will lead to rewetting. Experience has revealed that rewetting of coffee lots, particularly

during the later stage of drying, leads to greater fungal incidence compared to initial stage of drying. Rewetting of beans was also found to increase drying duration leading to bleaching of coffee, especially during storage (Velmourougane and Bhat, 2009).

28.4.5 Moisture management

In Indian coffee farms, the most commonly practiced method for determination of moisture are 'biting' test and test weight methods (Venkatesh, 2000). Although moisture meters (Kappa, Sinar, etc) are used in large farms (>25 ha) and curing factories, small and marginal coffee farmers adopt conventional methods. In small and marginal farms, moisture is estimated based on water loss from the drying coffee mass using a metallic vessel called a 'forlit' (40 L) (Figure 28.7). When a forlit of raw (uncured) coffee weighs 15.5 kg (for parchment coffee) or 18 kg (for cherry coffee), the moisture content in raw coffee is estimated to have reached the appropriate drying level of 10% and 11%, respectively. However, this method does not ensure the required moisture levels in green coffee (Gopinandhan *et al.*, 2008b).

Based on the constant volume study, Velmourougane *et al.* (2006b) showed that moisture
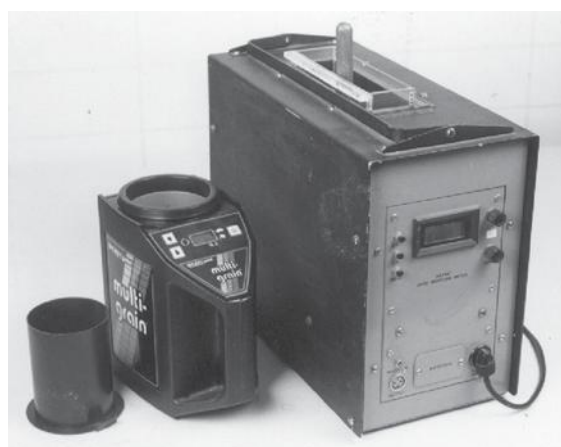
Figure 28.7   Methods of moisture measurement in coffee beans at 'on-farm' level in India.

estimation through test weight method did not always provide accurate results compared to moisture meters and oven-dry method (Figure 28.8). Coffee measured by the test weight method was either under- or over-dried, affecting the cup quality and attracting higher mold and OTA contamination.

28.4.6   On-farm storage

A clear-cut separation between different lots of green coffee beans (processed or dried during various stages) requires to be maintained during storage to retain the uniqueness of individual lots with respect to overall quantity and quality

(Panneerselvam *et al.*, 2006). Adequate care must be taken to ensure that coffee stacks do not tilt towards the walls of the godowns (underground storage buildings), which occasionally might harbor toxigenic molds. Stacking of raw coffee requires wooden barriers prepared from parchment husk filled in gunny bags, while clean coffee stacks should be built from wooden planks. To prevent cross-contamination, coffee godowns should be rodent-proof and free from any volatile materials (e.g. fertilizers, pesticides, petroleum products, etc). It is also advisable to transport coffee for curing before the onset of the monsoon season, as warm and humid conditions tend to favor the rapid growth of spoilage and toxigenic molds.
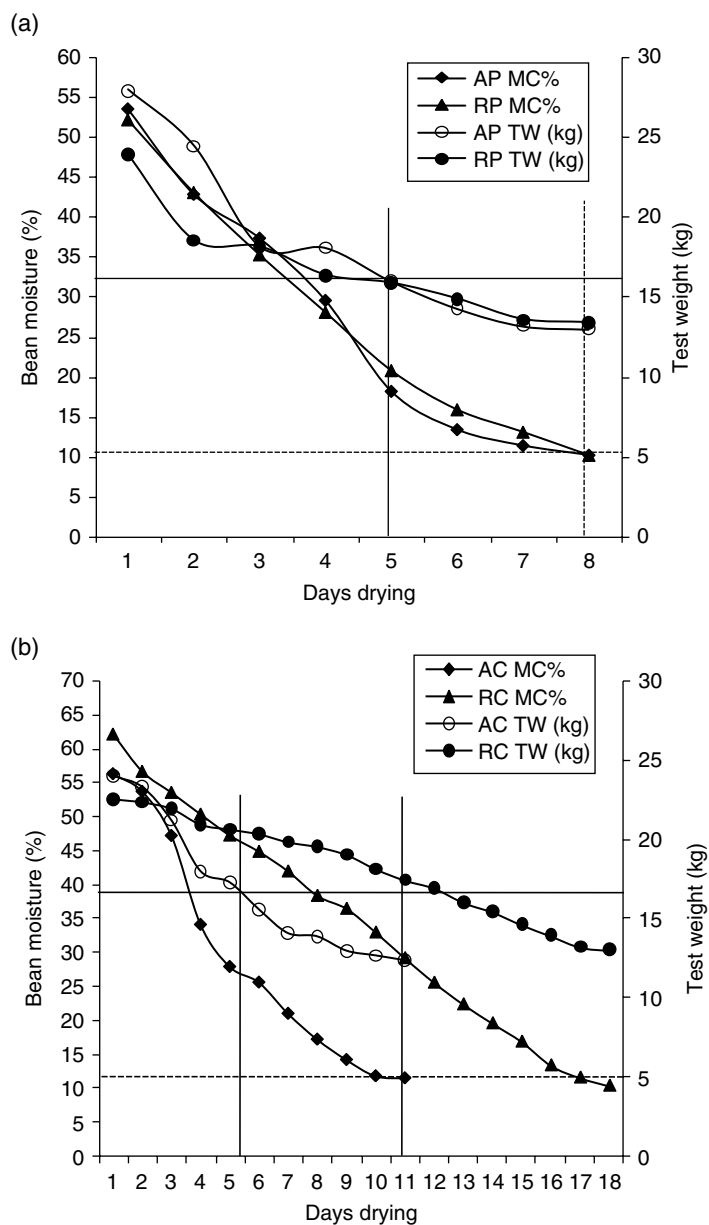
Figure 28.8 Comparison of moisture measured through moisture meter and forlit for (a) parchment and (b) cherry. AP and RP: *Arabica* and *Robusta* parchment; AC and RC: *Arabica* and *Robusta* cherry; MC: moisture content; TW: test weight (Forlit weight). After Velmourougane *et al.* (2006a).

28.5 Coffee waste management and OTA contamination

There are two major waste products of coffee processing: solid waste (coffee pulp/skin) and liquid waste (processing wastewater). Generally, solid wastes are not considered a major pollutant due to its use as compost in coffee farms (Kurian and Velmourougane, 2011). However, it was found that coffee wastes, if not completely composted, can harbor various toxigenic molds which can survive on the partially composted material present in the vicinity. Based on a field study, Velmourougane et al. (2012) reported that composting coffee wastes may be a potential source of OTA contamination in coffee plantations. It was observed that OTA contamination occurred at a level of 8.4 and 14.24 ng g⁻¹ in naturally and artificially contaminated coffee husk subjected to composting. From this work, a minimum distance between the drying yard and composting area was recommended to prevent the spread of OTA molds onto coffee.

28.6 Curing factories as a source of OTA contamination

28.6.1 Dust control in curing factories

In curing factories, dust accumulation can be a potential source of mold contamination. From a study carried out at curing works during the peak curing activity, it was found that a higher quantum of dust was generated by the huller followed by the polisher, with the mold spores higher on the polisher, followed by the destoner and huller (Velmourougane et al., 2008b). Dust particles accumulated over an extended period of time were also found to harbor a high concentration of toxigenic molds. Provisions should therefore be made in curing works to avoid dust particles settling on curing machineries, in order to prevent cross-contamination by toxigenic molds. Coffee cherry husk is also reported to be a potential

carrier of A. ochraceus (Gopinandhan et al., 2006). OTA at levels of 3.6–53 ppb has been recorded in coffee cherry husk; effective separation and immediate disposal of the coffee husk is vital to minimize OTA contamination in coffee beans.

28.6.2 Defective beans and OTA contamination

'Defects' is the collective name for undesirable particles found in bulk green coffee. Defects can include fruit tissues, foreign matter as well as various types of beans, or parts of beans referred to as browns, blacks, bits, and insect-damaged beans. In general, bean defects are caused by pest damage, faulty processing, or rainy conditions leading to poor fruit development. Results based on studies of lower grades of coffee have indicated that defect classes can contain high levels of OTA. It is envisaged that processing methods can also be one of the primary reasons for occurrence of defects in coffee. Panneerselvam et al. (2000a) have reported uncured coffee samples to harbour high A. ochraceus and OTA as compared to cured coffee samples in Arabica and Robusta varieties. The higher incidence of OTA contamination in coffee gleanings from coffee plantations and rejected fruits on coffee farms has been reported (Panneerselvam et al., 2000b). Gollucke et al. (2004) reported higher levels of OTA in defect beans as compared to sound coffee beans. Effective segregation of gleanings from main coffee lots was therefore recommended as good agricultural practice (GAP). Coffee beans with sour defects were reported to have higher OTA levels compared to defects (Taniwaki et al., 2007).

Reports are available describing how spores of A. ochraceus, Penicillium chrysogenum, and Verticillium sp. were recovered from coffee berry borer (CBB) in coffee plantations (Vega et al., 1999). The association of CBB with Fusarium sp. and Paecilomyces lilacinus has also been reported (Perez et al., 1996; Posada et al., 1998). Velmourougane et al. (2010a) reported how the coffee berry borer acts as a vector of OTA mold in coffee and concluded that CBB-affected beans must be effectively sorted out.

28.6.3   Shipment

Being highly hygroscopic, coffee beans can readily absorb atmospheric moisture and this poses a serious problem during shipment over long distances. Adequate care should therefore be taken, using air-tight containers which are free of holes or corrosion with intact door locks and ensuring that storing (packaging) bags are free of possible leaks, wetting, or stains. 'Saddle stow' is the best method of stowing bags in a container as this can minimize air circulation between bags and reduce the transport of moisture to cold spots.

28.7   Application of GAP/GMP and HACCP principles

28.7.1   HACCP, food hygiene and food safety

Traditional food safety assurance systems such as good hygienic practices (GHP) and good manufacturing practices (GMP) rely on: (1) performance during procurement of raw materials, processing and manufacturing, storage, transportation, and distribution and (2) performance

undertaken to ensure that food produced are safe (end-product testing).

However, food industries and public health authorities have recently realized the limitations of this approach. The suggested codes only provide broad guidelines, and cannot be specific to a food or process development. The possibility exists that measures essential for food safety are not adhered to. The provision of codes is mainly based on experience, rendering it difficult to supervise the complexity. In addition, the code does not provide suitable mechanisms for identifying control measures which are essential for product safety.

Today's approach towards ensuring food safety is based on fulfillment of GMP and GHP and proper application of HACCP, which balances with GMP/GHP. Application of HACCP can ensure that potential hazards are systematically analyzed in food industries for production, processing, and manufacturing. HACCP complements the Codes of General Principles of Food Hygiene. Currently, application of HACCP approach has become compulsory in the EU and in the USA for various food products such as fish, meat, poultry, and their products (Table 28.1).

Table 28.1   Basic principles of the HACCP system.

	Principles	Steps
1	Conduct a hazard analysis	Identify hazards and assess the risks associated with them at each step in the commodity system. Describe possible control measures.
2	Determine the critical control points (CCPs)	A critical control point is a step at which control can be applied and is essential to prevent or eliminate a food safety hazard, or reduce it to an acceptable level.
3	Establish critical limit(s)	Each control measure associated with a CCP must have an associated critical limit which separates the acceptable from the unacceptable control parameter.
4	Establish a monitoring system	Each control measure associated with a CCP must have an associated critical limit which separates the acceptable from the unacceptable control parameter.
5	Establish corrective actions	A procedure for corrective action; indicates a deviation from an established critical limit when monitoring a CCP
6	Establish verification procedures	Such procedures include auditing of the HACCP plan to review deviations and product dispositions, and random sampling and checking to validate the whole plan
8	Establish documentation	Establish documentation concerning all procedures and records appropriate to these principles and their application

28.7.2   Code of good practices for OTA prevention in coffee production

The HACCP system can be viewed as a decision tree where critical control points (CCPs) can be identified along the product chain. At these CCPs, appropriate control measures are implemented to ensure the safety of the product. Based on field studies from farm to market, certain CCPs were identified along the coffee production chain (Table 28.2). Various measures to be adopted in order to manage/prevent molds or OTA during the production chain are listed in Table 28.3.

Table 28.2   Coffee processing chain and critical control points for OTA prevention. (GAP: good agricultural practices; GMP: good manufacturing practices; GHP: good hygienic practices, HACCP: hazard analysis and critical control point; CCP: critical control point)

	Wet processing (Parchment coffee production)			Dry processing (Cherry coffee production)		
	Process	GAP/GMP/GHP	HACCP	Process	GAP/GMP/GHP	HACCP
I. Production						
1	Harvesting	✓		Harvesting		CCP1
II. Processing						
1	Sorting		CCP1	Sorting		CCP2
2	Separation of floats	✓		Separation of floats	✓	
3	Pulping	✓		-		
4	Fermentation	✓		-		
5	Washing	✓		-		
6	Tray drying	✓		-		
7	Drying floors	✓		Drying floors	✓	
8	Thickness		CCP2	Thickness		CCP3
9	Stirring frequency		CCP3	Stirring frequency		CCP4
10	Moisture management		CCP4	Moisture management		CCP5
11	Winnowing/Cleaning	✓		Winnowing/Cleaning	✓	
12	Onfarm storage		CCP5	Onfarm storage		CCP6
13	Transport	✓		Transport	✓	
III. Curing factories						
1	Initial moisture	✓		Initial moisture	✓	
2	Drying	✓		Drying	✓	
3	Curing	✓		Curing	✓	
4	Dust contamination	✓		Dust contamination	✓	
5	Sorting (defective beans)		CCP6	Sorting (defective beans)		CCP7
6	Waste disposal		CCP7	Waste disposal		CCP8
7	Storage		CCP8	Storage		CCP9
8	Transport	✓		Transport	✓	
IV. Export (Shipment)						
1	Moisture (water activity)		CCP9	Moisture (water activity)		CCP10
2	Temperature		CCP10	Temperature		CCP11
3	Packing		CCP11	Packing		CCP12

Table 28.3 Code of good practices for OTA prevention in coffee production

Production steps/ processing chain	Do	Don't	Findings	References
Harvesting	Use of harvest mats to avoid fruit contact with soil	Processing of fallen fruits (gleanings) along with good coffee lot	Use of harvesting mats for coffee picking was found to decrease the OTA contamination in final coffee.	Panneerselvam *et al.* (2000b); Velmourougane and Bhat (2009)
			Coffee cherries picked from soil (gleanings) were found to harbour higher OTA-producing molds and OTA	
Sorting	Sorting of coffee cherries before processing	Processing of bulk fruits	Sorting coffee cherries into different maturity categories before drying was found to lower the OTA mold incidence compared to drying	Panneerselvam *et al.* (2002); Naidu *et al.* (2005); Velmourougane and Bhat (2009)
Processing	Adjustment of pulper and washer to avoid fruit injury		Fruit injury and pulp contamination at processing level was found to favor OTA mold proliferation in the processed coffee	Silva *et al.* (2000); Panneerselvam *et al.* (2001)
	Timely processing of harvested cherries		Delays between harvesting the coffee cherries and the onset of processing increases the risk of contamination by ochratoxin-producing molds and OTA in coffee, leading to production of unhygienic coffee with poor cup quality.	Velmourougane *et al.* (2011b)
	Following optimum fermentation conditions		Fermentation step was reported to reduce ochratoxigenic mold incidence and OTA contamination in coffee beans along with improvement in cup quality.	Masoud and Kaltoft (2006); Shetty and Jespersen (2006); Angioni *et al.* (2007); Velmourougane *et al.* (2008a, 2011a, 2012)
Drying floors	Drying coffee on concrete surfaces	Drying coffee on soil- or cowdung-smeared surfaces	Better quality of coffee with minimal mold and OTA contamination could be obtained by drying the coffee cherries on tarpaulin and cement surfaces rather than drying directly on soil surface.	Velmourougane *et al.* (2006a, 2010b)

Production steps/ processing chain	Do	Don't	Findings	References
Drying thickness	Optimum drying thickness		Better quality of coffee with minimal mold and OTA contamination could be obtained by drying parchment coffee at 4 cm and cherry coffee at 6 cm	Naidu *et al.* (2005)
Stirring frequencies	Optimum turning of coffee		Better quality of coffee with minimal mold and OTA contamination could be obtained with a minimum of 4 rakings/day	Velmourougane *et al.* (2010b)
Moisture management	Use of calibrated moisture meters	Dependency on biting method and forlit method	Coffees measured through test weight method were either under dried or over dried affecting cup quality and attracting higher mold and OTA.	Venkatesh (2000); Velmourougane *et al.* (2006b); Gopinandhan *et al.* (2008b)
On-farm storage	A clear-cut demarcation between different lots of green coffee beans (processed/ dried at various stages)	Bulk storage and under dried coffee storage	Bulk storage was reported to spoil quality as well as being prone to mold attack	Panneerselvam *et al.* (2006)
Defective beans (cuts, bits, blacks, berry borer damaged)	Effective segregation of defective beans before storage	Storing bulk coffee	Coffee berry borer can act as a vector and can carry the Mycotoxin-producing fungal spores onto the coffee beans.	Perez *et al.* (2003); Vega *et al.* (2006)
			A. ochraceus infection level of 4–12% and OTA level of 2–24 ppb were observed in the borer infested beans	Velmourougane *et al.* (2010a)
Waste disposal	Maintain a minimum distance between composting yard and coffee processing area	Disposal of coffee wastes nearing processing unit	OTA contamination to a level of 8.4 and 14.24 ng g^{-1} was recorded in composting coffee wastes	Velmourougane *et al.* (2012); Gopinandhan *et al.* (2006).
	Remove dust particle settling on the curing machines to prevent contamination by molds and mycotoxins	Unhygienic machineries	Dust accumulated over a period of time was also found to harbor a high percentage of toxigenic molds	Velmourougane *et al.* (2008b)

(*Continued*)

Table 28.3   (Continued)

Production steps/ processing chain	Do	Don't	Findings	References
Storage at curing factories	A clear-cut demarcation between different lots of green coffee beans (processed/dried at various stages)	Bulk storage	Bulk storage was reported to spoil quality as well as being prone to mold attack	Panneerselvam *et al.* (2000a)

28.8   Conclusions and future outlook

Based on the available reports, it is evident that coffee is highly susceptible to contamination by toxigenic molds which are able to produce mycotoxins. The susceptibility is present along the entire processing chain, which can lead to major economic losses to the producing country. Hygienic and good manufacturing practices are pre-requisites to be followed at each step of processing for the production of high-quality coffee. Based on scientific studies, moisture management in coffee production chain was found to be a major determining factor on OTA contamination in coffee. Adoption of GAP/ GHP/GMP and HACCP principles to prevent mold and OTA contamination in coffee are therefore essential.

Future investigations into alternative methods of non-thermal or non-chemical mode of preservation (such as modified atmospheric packaging, ionizing radiations), along with application of hurdle technology (such as metabolic exhaustion of microorganisms, stress reactions, homeostasis, multi-target prevention), are required to ensure the safety and quality of coffee beans. As a policy for production of quality OTA-free coffee, quality control departments have to pre-certify that beans are free of OTA contamination, especially those requiring export for longer distances. In addition, local governments need to establish awareness among local growers, plantation workers, processors, and consumers by conducting education campaigns relevant to codes of good practice for production of OTA-free coffee. In addition, providing coffee growers with basic farming facilities (drying yard, hygienic environment, etc.) at subsidized rates and fixing premium prices for high-quality products will encourage the growers to adopt codes of good practice.

Acknowledgements

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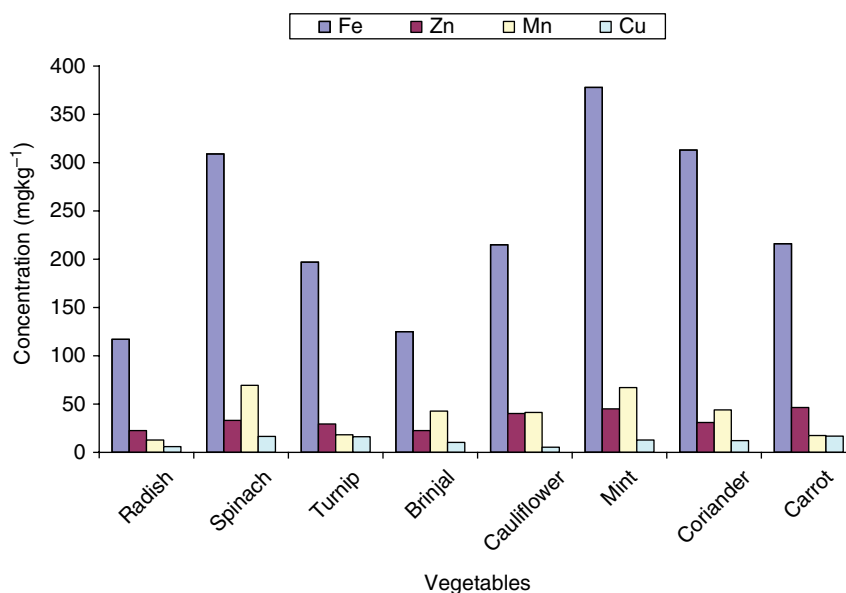
References to tables are given in bold type.

Plate 13.1   Heavy metal content (mg kg^{-1} dry weight) in plants grown in wastewater-irrigated soils. Scientific names of plants: radish: *Raphanus sativus*; spinach: *Spinacia oleracea*; turnip: *Brassica rapa*; brinjal: *Solanum melogena*; cauliflower: *Brassica oleracea* var. botrytis; mint: *Mentha requienii*; coriander: *Coriandrum sativum*; carrot: *Daucus carota*. Adapted from Arora *et al*. (2008).
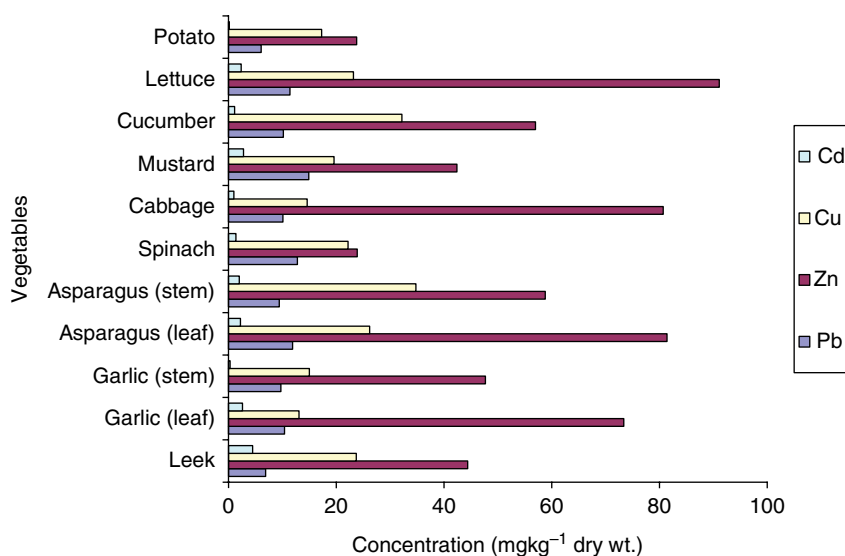
Plate 13.2 Mean concentrations (mg kg^{-1} dry weight) of heavy metals in some vegetables sampled from Chongqing markets, China. Adapted from Yang *et al.* (2011).
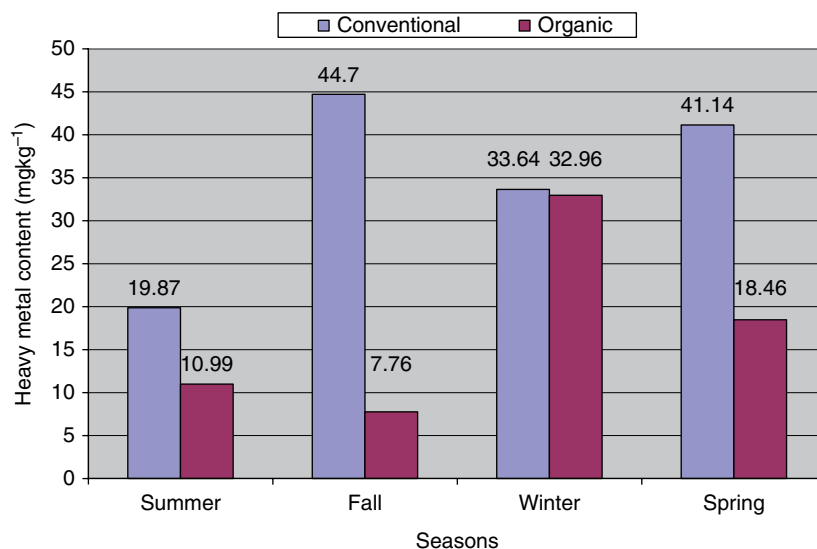
Plate 13.4 Seasonal variation of heavy metal content in conventionally and organically farmed potato tubers collected from Egyptian markets. Data adapted from Mansour *et al.* (2009b).
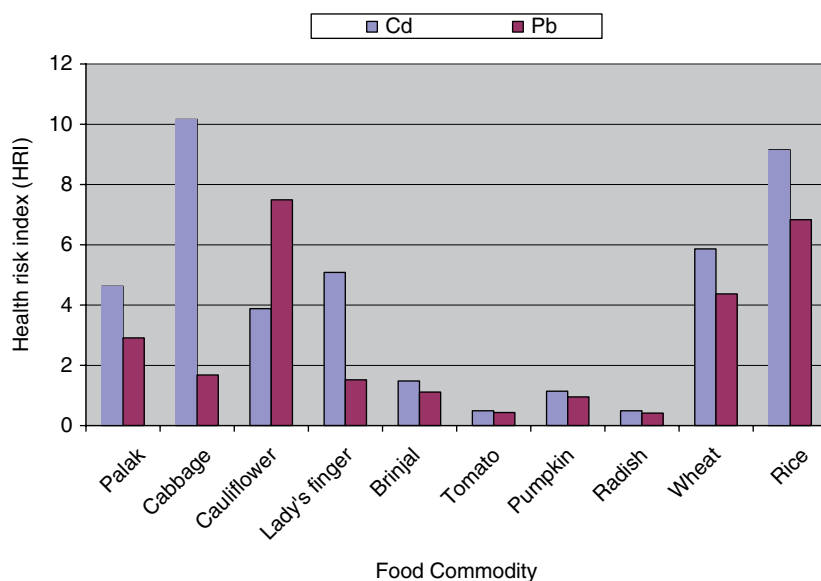
Plate 13.5 Health risk assessment for cadmium and lead via intake of foodstuffs from wastewater-irrigated sites in India; based on estimated health risk index (HRI). HRI≥1 indicates a health risk to humans. Analyzed vegetables and cereal crops: palak: *Beta vulgaris* L.; cabbage: *Brasssica oleracea* L.; cauliflower: *Brassica oleracea* L.; lady's finger: *Abelmoschus esculentus* L.; Brinjal: *Solanum melongena* L.; tomato: *Lycopersicon esculentum* L.; pumpkin: *Cucurbita maxima* Duch.; radish: *Raphanus sativus* L.; wheat: *Triticum aestivum* L.; rice: *Oryza sativa* L. Adapted from Singh *et al.* (2010).
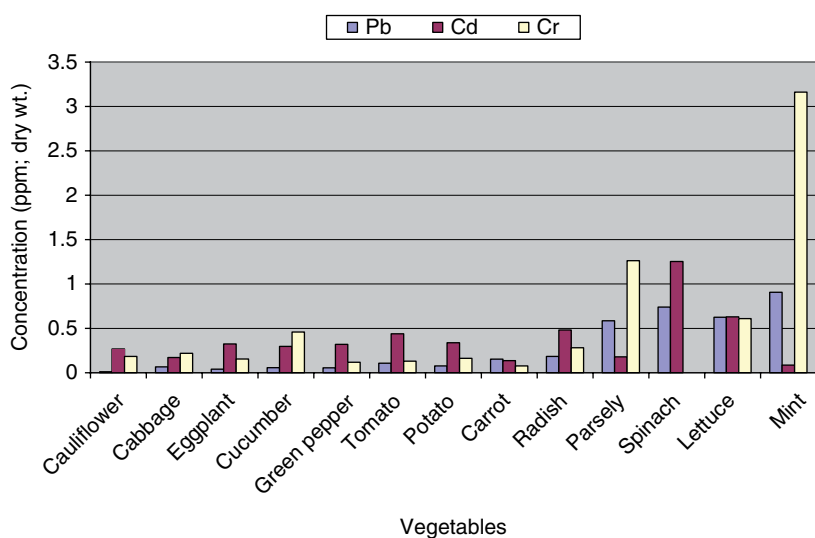
Plate 14.1 Concentration levels (ppm dry weight) of selected heavy metals in different vegetable samples grown and sold in Lebanon. Adapted from Al-Chaarani *et al.* (2009).
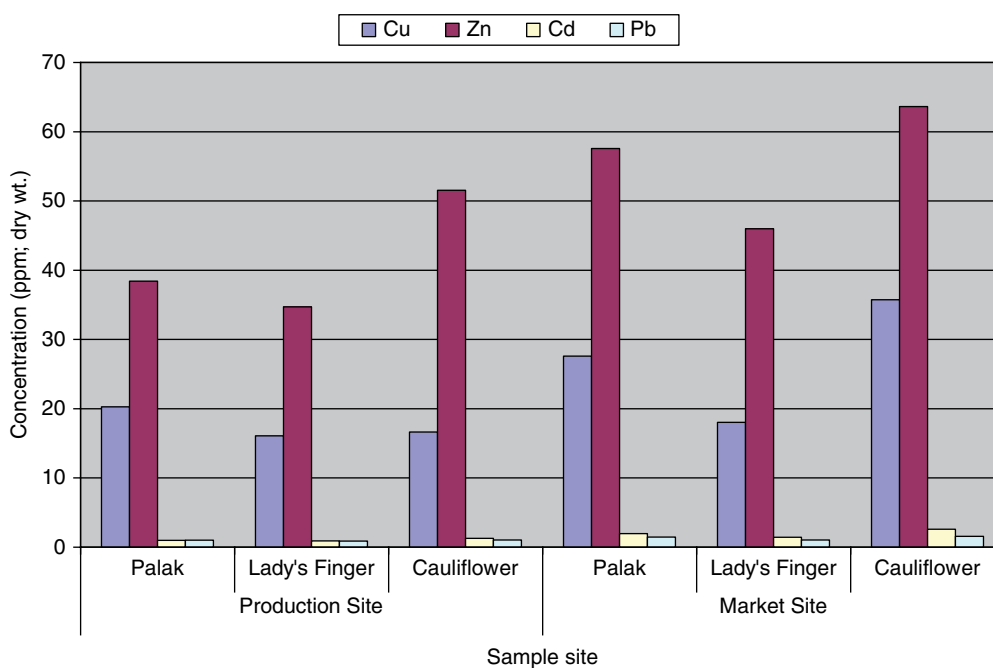
Plate 14.2 Mean concentrations (μg g^{-1} dry weight) of some heavy metals in selected vegetables from production and market sites of Varanasi, India. Vegetables: palak (*Beta vulagaris*); lady's finger (*Abelmoschus esculentus*); cauliflower (*Brassica oleracea*). Adapted from Sharma *et al.* (2009).
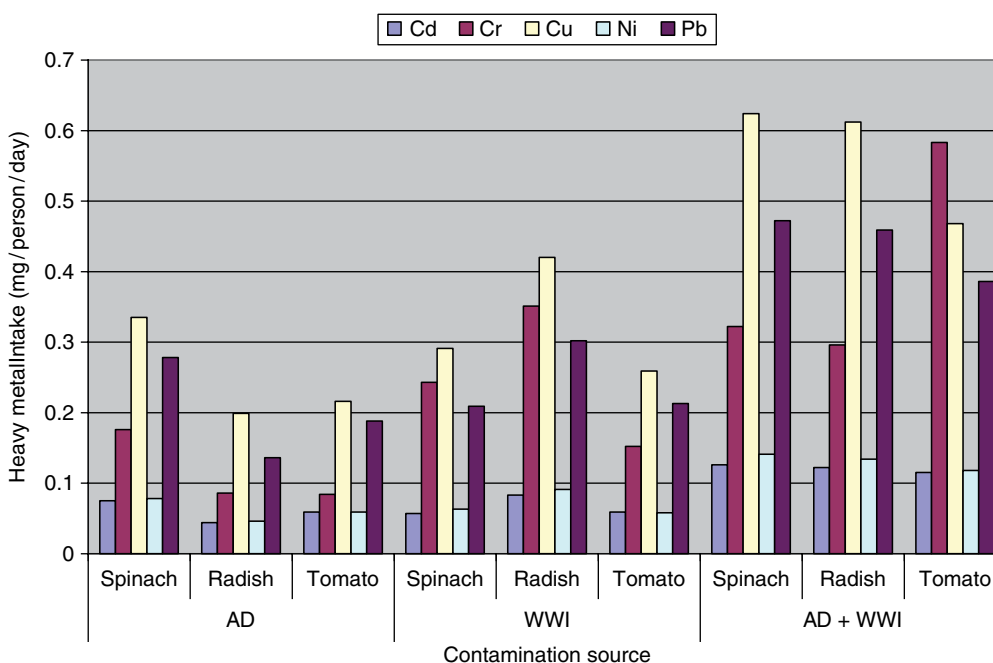

Plate 14.3 Daily intake of heavy metals (mg/person/day) in different vegetables as influenced by atmospheric deposition (AD), waste water irrigation (WWI) and a combination of these (AD+WWI). Adapted from Pandey *et al.* (2012).

Plate 14.4 Concentration of blood-Pb in urban children and adults in different areas. Adapted from Fewtrell *et al.* (2004). Countries involved in each region: Africa-I: Nigeria; Africa-II: South Africa; America-I: Canada, United States; America-II: Argentina, Brazil, Chile, Jamaica, Mexico, Uruguay, Venezuela, Ecuador, Nicaragua, Peru; Eastern Mediterranean-I: Saudi Arabia; Eastern Mediterranean-II: Egypt, Morocco, Pakistan; Europe-I: Denmark, France, Germany, Greece, Israel, Sweden; Europe-II: Turkey, Yugoslavia; Europe-III: Hungary, Russian Federation. South-East Asia-I: Indonesia, Thailand; South-East Asia-II: Bangladesh, India; Western Pacific-I: Australia, Japan, New Zealand, Singapore; Western Pacific-II: China, Philippines, Republic of Korea.
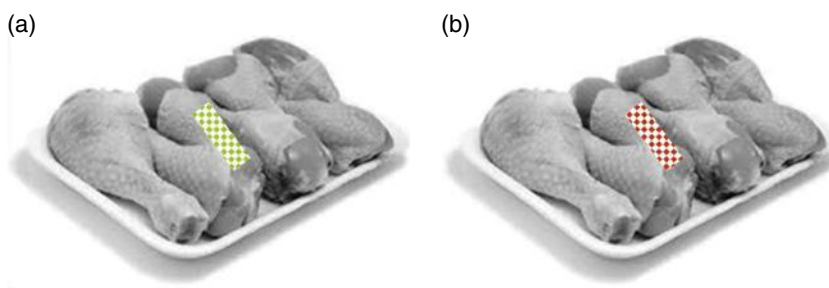

Plate 17.4 Colorimetric nanosensor for bacterial growth. When the produce is fresh the label color is (a) green; when the product is contaminated the label color (b) changes to red, communicating risk to consumers or retailers.
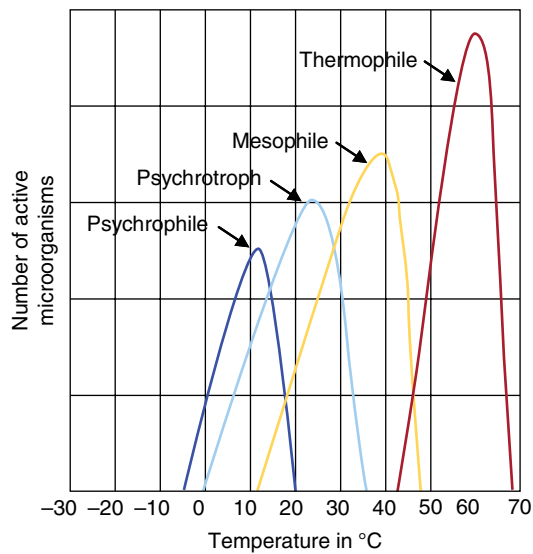
Plate 20.2 Growth rate of different groups of microorganisms in response to storage temperature (source: HEA, 2009 with kind permission)
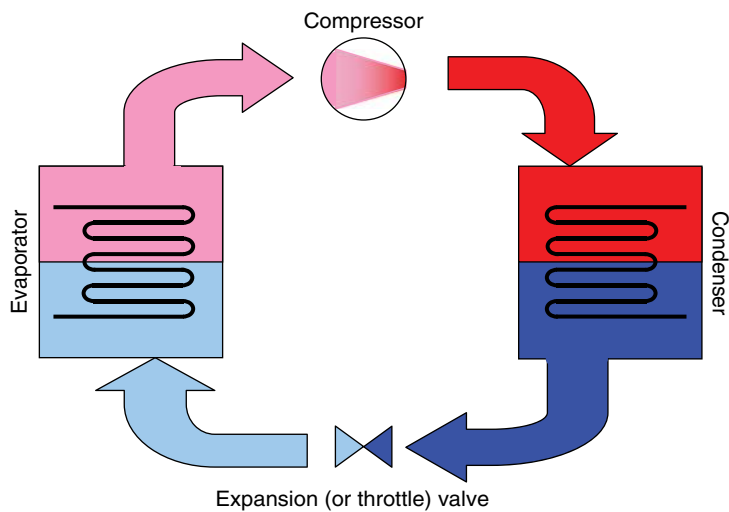

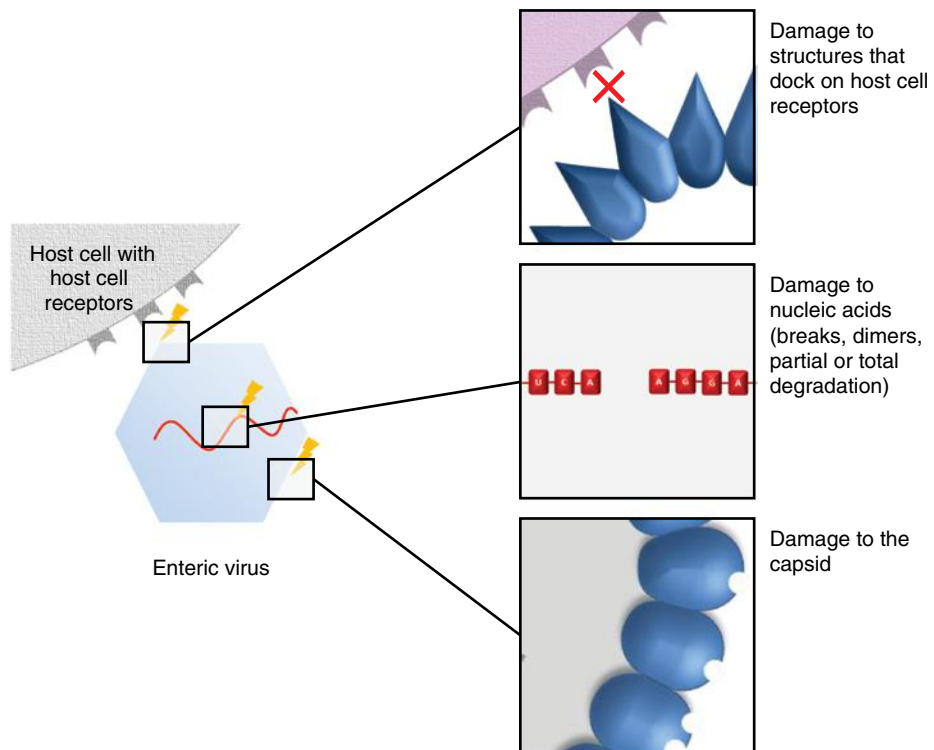**Plate 20.4** Principle and components of a vapour-compression refrigeration set

Plate 23.1   Overview of inactivation mechanisms of viruses

Plate 28.3 Coffee berries during different maturity stages: (a) greens; (b) half-ripes; (c) ripes; (d) over-ripes; (e) tree-dried; and (f) bult lot. After Velmourougane *et al.* (2006a).