Handbook of Laboratory Animal Management and Welfare

Sarah Wolfensohn and Maggie Lloyd

Fourth Edition



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Dedication

This book is dedicated to the staff of the Research Animals Department at the RSPCA, in recognition of the contribution they have made towards improving the welfare of laboratory animals.

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Handbook of
Laboratory Animal Management and Welfare

Fourth Edition

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Chapter 1

Education and Training for the Personal Licence Holder

Introduction

The use of animals in research in the UK and Europe is controlled by several pieces of legislation, namely Council of Europe Convention ETS 123, EU Directive 2010/63 and the UK Animals (Scientific Procedures) Act 1986. These require that institutions where such research is carried out must have sufficient numbers of suitably trained staff. EU Directive 2010/63 specifies training requirements for four categories of personnel: those carrying out procedures on animals, those designing procedures and projects, those taking care of animals and those killing animals[1]. The framework places clear responsibilities on persons in these roles. These are key functions, and it is imperative that people carrying out these duties are competent, to ensure that animal welfare and scientific integrity are maintained. People intending to carry out these roles must complete appropriate training prior to starting work. In addition, the framework requires that persons carrying out procedures, taking care of animals or killing animals must be supervised until they are competent. The supervisor retains this responsibility until training is completed and the requisite competence demonstrated.

Any person wishing to embark on a career in biomedical research must appreciate that they will become primarily responsible for the welfare of the animals on which they have performed scientific procedures. It is an important part of that responsibility that the procedures are performed competently, causing the least amount of trauma to the animals. It follows that those who are carrying out procedures need to understand the legal and ethical framework under which experiments may be performed, how to handle and take care of animals and how to carry out the procedures. It is important for animal welfare and for good science that procedures are carried out to the highest standards, using the best techniques and equipment available, to minimise animal suffering and maximise scientific output. This is usually achieved by a period of fundamental education and training, followed by a period of practice under supervision, in order to acquire the requisite knowledge, skills and attitudes to become competent.

Those who take responsibility for the direction and control of a programme of work must be able to manage such a programme, taking all opportunities for Reduction, Refinement and Replacement (the three Rs) and encouraging and promoting an appropriate attitude in those working in the programme. Again, specific training may be required for this role, for example in experimental design and project planning, in communication and in team building. They may also be required to communicate with lay audiences to promote public understanding of science, and training for this may be necessary.

Those who have responsibility for the care of animals must understand the needs of the species they are working with, and know how best to provide for these needs in a laboratory environment.

Those who are just killing animals for tissues must be able to perform the chosen methods of killing swiftly and competently, causing a minimum of distress to the animal and maintaining the carcass such that it is appropriate for use in the subsequent experiment.

In addition, those personnel with responsibility for overall management of facilities must be in a position to maintain the fabric of the establishment and ensure adequate staffing, and put management systems in place for the prevention of unauthorised procedures. They need to develop a culture of care in the institution and must ensure that appropriate training is available for all staff. These people need to be aware of the legal and ethical issues surrounding the use of animals in scientific procedures, and also have training needs. Those acting as named veterinary surgeons or named animal care and welfare officers may also need to undergo specific training for the role.

Education involves the acquisition of relevant knowledge and development of a suitable attitude, whereas training involves the acquisition of manual skills. Both of these are important in the training of those about to embark in a career in biomedical research.

EU Directive 2010/63 states that EU member states should publish guidelines on training requirements based on the following key areas.

1. National legislation in force relevant to the acquisition, husbandry, care and use of animals for scientific purposes.

2. Ethics in relation to the human–animal relationship, intrinsic value of life and arguments for and against the use of

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animals for scientific purposes.

3. Basic and appropriate species-specific biology in relation to anatomy, physiological features, breeding, genetics and genetic alteration.

4. Animal behaviour, husbandry and enrichment.

5. Species-specific methods of handling and procedures, where appropriate.

6. Animal health management and hygiene.

7. Recognition of species-specific distress, pain and suffering of most common laboratory species.

8. Anaesthesia, pain-relieving methods and killing.

9. Use of humane end points.

10. Requirement of Replacement, Reduction and Refinement.

11. Design of procedures and projects, where appropriate.

This list is not exhaustive: additional training in other areas may also be required, and those working with animals in research have a responsibility to determine their individual training needs and ensure that these are met.

Framework for Education and Training of Licence Holders

In 2012 an Expert Working Group (EWG) was set up by the European Commission to develop a common education and training framework for the EU to fulfil the requirements under Directive 2010/63/EU. The EWG defined the fundamental principles, set the common framework and identified output-driven quality standards for contents. The framework should be implemented through accreditation of training modules, assessment of competence, oversight of the delivery of training and identification of additional training needs. The framework is not binding, and each Member State can interpret whether and how this general guidance is to be implemented. At the time of writing this guidance is under development. The Federation of European Laboratory Animal Science Associations (FELASA)[2] and the Animal Procedures Committee[3] have also published reports detailing recommendations for the education and training of persons involved in animal experiments. The core competencies of these persons have been defined as follows[2].

- To be aware of European and national laws and guidelines relating to the conduct of experimental or other scientific procedures on animals.
- To be aware of and to respect societal ethics in relation to animal research.
- To understand and respect the general rules of the animal facility, where procedures are carried out.
- To understand the theoretical background of tasks one is expected to do, so as to safeguard animal well-being and ensure the relevance of scientific findings.
- To be competent in handling and other techniques one is expected to carry out.
- To be able to recognise pain and discomfort and to assess the welfare status of animals with which one is working.
- To be aware of the need for, and capable of taking, appropriate action when adverse outcomes occur during or following procedures.
- To be knowledgeable concerning the uses of laboratory animals and be competent to take appropriate measures to minimize interfering factors when a procedure is conducted.

These can be considered to be the core competencies required by those undertaking experiments on animals, and any training programme should address these areas. The reports describe a suitable curriculum for such training, and suggest ways in which competence can be achieved. Learning can be achieved through the provision of a variety of learning opportunities, including lectures, tutorials, discussions, videos and reading, but practical experience is particularly important.

The EWG has identified that the training framework should be flexible, available and accessible, affordable and of agreed quality to ensure competence of staff and facilitate free movement of personnel. Training is divided into individual modules, some of which (termed core modules) are required for staff in all four categories, and others of which are required only for specific functions (termed prerequisite and additional modules). There may be other modules developed to cover issues of national relevance, such as other legislation relevant to the use of animals for scientific purposes, or specialised skills. It is also expected that there will be ongoing lifelong learning, and researchers will be expected to keep up to date with new developments, such as new techniques, and advances in the three Rs. Institutions will be expected to have a training officer, whose role will be to ensure that staff are educated, competent and continuously trained.

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For each module, the framework details the desired learning outcomes, and the theoretical knowledge and practical skills to be obtained, define how the module should be assessed and identify pass/fail criteria. The focus for training will be the development of competence: passing the required modules for a task should indicate that the individual has attained a level of learning appropriate for the performance of the task, but competence will be attained only after a period of supervision following initial training.

Some of the training modules are specific to a species or group of species, and initial training may need to be completed fully for one or more species or groups. Extension of training to add further species will require demonstration of attainment of learning outcomes for the new species, but may not always require all the elements of the initial training module for the new species to be completed, as there may be common elements which do not need to be repeated. Full details of the requirements can be found on the UK Home Office website (www.homeoffice.gov.uk).

Core modules

Modules to be completed by personnel in all four categories include:

- national legislation relevant to animal care and use,
- ethics, animal welfare and the three Rs,
- basic species-specific biology (including routine animal husbandry and enrichment practices),
- animal care, health and management,
- recognition of species-specific pain, suffering and distress including humane end points,
- humane methods of killing (theory).

Prerequisite and additional modules

Modules to be completed by personnel according to their function and individual needs include:

- more detailed consideration of ethics, animal welfare and the three Rs,
- detailed animal husbandry, care and enrichment practices,
- animal handling methods,
- minor procedures,
- anaesthesia and analgesia,
- humane methods of killing (skills),
- invasive techniques including surgery,
- design of procedures and projects.

Education and training in the UK

Since 1994 in the UK, applicants for personal licences allowing them to carry out scientific procedures on animals have been required to complete a training programme, accredited by a recognised body according to a syllabus set by the Home Office[4]. The Society of Biology, the Scottish Accreditation Board and the Universities Accreditation Scheme are currently recognised by the Home Office for this purpose.

Aims of Accredited Training

When learning any new skill there is an initial period of unconscious incompetence[5], during which the person is not aware of the skills required to carry out the new task, and may not be aware that they have a deficiency in this area. This is followed by conscious incompetence, when the person becomes aware of their deficiency and makes a commitment to learn and practise the new skill. They then move to the conscious competence stage, when they can perform it reliably at will but need to concentrate and think in order to perform the skill. Finally, they reach the stage of unconscious competence, when the skill becomes so practised that it becomes second nature. The person might then be able to supervise and teach others.

It is not expected that anyone completing accredited training will immediately demonstrate a high level of competence in the skill areas identified above. The person must become conscious of their incompetence before development of the new skill or learning can begin, and the aim of accredited training is simply to move the person into the conscious incompetence stage, so that

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they become aware of the range of skills they need to develop to become competent. The object of mandatory training courses is therefore to establish a sound foundation upon which competence can be effectively built, and a period of on-the-job training under the supervision of someone already expert and up to date in the field will be required subsequently until practical competence is achieved. New researchers should take an active role in their training, and liaise closely with their supervisors to devise a personal development plan, to ensure that they receive the support required to become competent.

Continuing Professional Development and Reflective Practice

New researchers are 'loaded up' with the requisite knowledge regarding the biological and behavioural needs of animals. It is impressed upon them that they have a personal responsibility to implement the three Rs. They are shown how to carry out procedures correctly and told where to seek guidance. However, techniques and methods are constantly improving and, in order to keep up to date, the experimenter needs to reflect on their own actions and actively seek improved methods. Training is often approached like a battery[6] – charging students up with knowledge, which they discharge when they enter the world of practice – yet this is quite inappropriate in a fast-changing world. EU Directive 2010/63 requires that personnel are 'educated, competent and continuously trained', introducing a requirement for personnel to undergo continuing professional development (CPD). Experimenters may leave their training sessions with the latest information, but unless this is accompanied by an acceptance that this knowledge is likely to be superseded, and the drive to seek actively for up-to-date information, they will not identify and implement refinements as they develop.

Training programmes should encourage participants to reflect on their actions, and to consider how they can improve their techniques to improve both animal welfare and the quality of science. CPD is now a requirement for biomedical scientists. New researchers therefore need to be trained in factual knowledge and practical skills at the start of their careers, but then need to implement reflective practice, where they become self-critical and actively seek the advice of others, to determine their own ongoing CPD needs. Supporting this reflective practice is an important role for the supervisor.

Summary

Becoming a biomedical researcher carries with it a responsibility for learning a set of new skills. This is accomplished by foundation training followed by period of supervision until competence is achieved. This needs to be backed up by a commitment to ongoing reflective practice and CPD to ensure that skills continue to develop, to maintain animal welfare. A person should only become a researcher if they are prepared to accept the responsibilities that come with the job.

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Chapter 2

Ethical Considerations around using Animals in Research

History of the Use of Animals in Research

From ancient times, people have strived to understand the workings of nature and the universe. Animal use in science dates back to Eristratus and Herophilus in the third century BC and Galen in the second century AD[1]. Roger Bacon published *Opus Majus* in 1265, in which he noted the causes of error: authority, custom, popular prejudice and the concealment of ignorance with the pretence of knowledge. He pointed out that the two methods of acquiring knowledge are argument and experience. He asserted that argument alone is not enough, for 'the strongest argument proves nothing so long as the conclusions are unverified by experience'. Bacon's insistence on the gathering of data is one of the hallmarks of modern science.

The birth of modern science dates to the year 1543 and the publication of Copernicus' *De Revolutionibus Orbium Coelestium* (On the Revolutions of the Heavenly Spheres) and Vesalius' *De Humani Corporis Fabrica* (On the Fabric of the Human Body), which challenged systems of belief dating back to the second century. Previously, medicine had been largely based on the teachings of Galen, whose knowledge of human anatomy was largely deduced from animal dissections (the dissection of human bodies was generally not accepted in ancient times), and whose treatment of disease was based upon the doctrine of the four bodily humours. Vesalius performed detailed dissections of the human body and spotted inaccuracies in Galen's descriptions. In challenging them he destroyed the foundation of medieval medical practice, which, like astronomy, was based upon ancient tradition and inherited knowledge. Ground-breaking experiments that demonstrated the circulation of the blood[2] would not be countenanced today owing to their method in animal use, yet underpin modern cardiovascular physiology.

As the use of animals in research increased, so too did opposition to vivisection. In 1789, utilitarian philosopher Jeremy Bentham wrote of animals 'the question is not, can they reason, nor can they talk, but can they suffer?', opening the debate on the ethics of animal use in experiments. Legislation governing the protection of animals has been in existence for many centuries. In the UK laws were passed against cruelty to animals late in the 17th century. In 1822, Martin's Act (after its sponsor Richard Martin) was passed, and primarily protected cattle and horses (it did not include cats, dogs or birds). It was amended in 1835 to protect all domestic animals. A Society for the Prevention of Cruelty to Animals was founded in 1824. Princess Victoria extended her patronage to the society in 1835, and it became the Royal Society for the Prevention of Cruelty to Animals (RSPCA). The subsequent rise of the anti-vivisection movement encouraged the development of self-regulation. In 1831 British physiologists attempted self-regulation with the publication of Marshall Hall's five principles, which were:

- an experiment should never be performed if the necessary information could be obtained by observations,
- no experiment should be performed without a defined, obtainable, objective,
- scientists should be well-informed about the work of their predecessors and peers in order to avoid unnecessary repetition of an experiment,
- justifiable experiments should be carried out with the least possible infliction of suffering (often through the use of lower, less sentient animals),
- every experiment should be performed under circumstances that would provide the clearest possible results, thereby diminishing the need for repetition of experiments.

Hall also proposed that results should be made available for public scrutiny. These principles bear a great deal of similarity to the three Rs, published 128 years later, and to current legislation. In 1871 the British Association for the Advancement of Science developed a moral code of practice but regrettably neither this, nor Hall's proposal, were readily adopted. In August of 1874 the use of two dogs in an experimental demonstration of epilepsy created uproar at a meeting of the British Medical Association in Norwich. At the meeting there were vociferous protests against the experiments, which were performed by the French physiologist Eugene Magnan. At one stage the president of the Royal College of Surgeons of Ireland cut the bindings holding one

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of the dogs and released it. The RSPCA later took Magnan and the three Norwich doctors who had arranged the demonstration to court, accusing them of unnecessary cruelty to the animals. In this first prosecution against the use of animals in experiments, the Norwich men were found not guilty because they did not perform the demonstration, and Magnan had returned to France. Nevertheless, the magistrates granted that the RSPCA was justified in bringing the action, and declined to award defence costs.

With opinions polarising, increasing levels of violent protests, especially since the 1960s, reinforced a bunker mentality as laboratories using animals closed their doors to visitors. Some breeding centres were forced to close, with the unintended consequence of increased transport times for animals[3]. The principles of Reduction, Refinement and Replacement (the three Rs) in animal experiments, proposed by Russell and Burch[4], took time to gain their now almost universal acceptance and for necessary improvements to follow, but the principles of the three Rs are implicit in the Animals (Scientific Procedures) Act 1986. All UK scientists are therefore legally obliged to use alternative approaches to the use of animals where possible, to use the minimum number of animals and to use protocols which cause the least pain, suffering or distress. The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) was established in 2004 and is an independent scientific organisation, tasked by the UK Government with supporting the UK science base through the application of the three Rs. It is also the UK's largest funder of three Rs research. The NC3Rs works in partnership with bioscience research funders, academia, industry, regulators and animal welfare organisations, both in the UK and internationally, to advance the three Rs. However, the welfare of laboratory animals continues to be strongly debated. Most observers agree that constructive dialogue has increased the quality of both animal welfare and research output, whereas simple hostility has been counterproductive. Attitudes to laboratory animals will undoubtedly alter in the future as our moral and cultural perspectives change.

The Ethics of Using Animals in Experiments

The word ‘ethics’ is used in many contexts, often incorrectly, leaving the reader confused as to its meaning. For the purposes of this book, it is an examination of the acceptability of the motives that drive the behaviour of people[5], whether they are scientists who use animals or people who stand up for animal rights. All use of animals for human benefit creates a dilemma. There is a spectrum of opinion on whether there is justification for using animals for our own ends: some people believe that animals have rights and it is wrong to use them, whether for food, in research, as beasts of burden, as pets or to kill them as vermin. Another group believes it is acceptable for humans to use animals in any way we think fit. Most people fall somewhere in between these two extremes.

The philosophical case for unimpeded use of animals for research is usually based around a concept of the superiority of humans over other species. When the research is for medical purposes the moral obligation of humans to prevent the suffering of other humans is also cited. Others argue that all living creatures have inherent value and hence moral rights. It would seem contradictory that a species could be similar enough to humans for experimental data to be useful, yet different enough for any suffering to be morally acceptable. Further, the moral status of a ‘subject of a life’ would include the right never to be treated merely as a means to the ends of others[6]. The justification for using animals in biomedical research depends on them being different from humans, with a reduced capacity to suffer (this in itself is the subject of much debate), whereas the validity of the results obtained depends on their physiological similarity to humans. The debate about animal experimentation has waxed and waned over the decades but it has never gone away, and some aspects of the debate are essentially unchanged from those used a century ago. An important factual point is whether or not animal experimentation has been essential in the development of important medical advances. Many abolitionist groups claim that animal research has never resulted in any medical benefits, that it has misled scientists, or that it is unnecessary because we have alternative non-animal techniques. Most doctors and scientists however, agree that animal research has played a major part in medical advances in the past and will continue to do so. Most researchers would like to see alternatives to animals developed, but most believe that animal research will continue to be necessary, at least for the foreseeable future. Other campaigners claim that animal research is cruel. There is no doubt that over 100 years ago it was cruel, before the advent of modern anaesthetic and analgesic drugs. But today things are very different: advances in veterinary science have made painless surgery possible, and everyone involved in animal research is very much aware of the need to maintain animal welfare. Research using animals can only be authorised provided the benefits outweigh the harms to the animals under a utilitarian framework with some additional restrictions. For example, work on developing cosmetic or tobacco products is not considered acceptable, no matter how much ‘benefit’ may be derived from the work. Similarly, work on great apes will not be authorised in the UK, for whatever purpose.

There is general agreement in the scientific community that progress through animal experimentation is expensive, bureaucratic and subject to animal-induced variability and that, aside from any ethical issues, it is rarely the method of choice. Extrapolation between species is a common cause of reducing confidence in experimental conclusions. However, these constraints alone are not

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enough to ensure that animals are only used when essential. Any justifiable use of animals must include an acceptance that animals are not merely tools. For many biomedical advances, animal research has indisputably been part of the pathway to progress, but that is not the same as saying that animal research was *necessary* for that progress. Indeed, some would say that advances have been made despite animal research. The crucial question is, how great are the benefits that *only* animal research can deliver?

Scientists who use animals would argue that an ability to balance or set aside the short-term price of agreeing to cause harm to animals for a long-term gain that is judged to be greater demonstrates moral responsibility, as they are acting as moral agents[7]. However, in order to perform statistically sound experiments animals usually need to be 'grouped' and this has an unintended effect of 'depersonalising' the group's members. In addition, when animals might suffer there is a natural tendency towards emotional detachment on the part of handlers and experimenters alike, for the protection of their own emotions. Both of these tendencies should be resisted in good laboratories by remembering the principle that 'good welfare results in good science' and must be based on understanding the individual as well as the group. Naturally, anti-vivisectionists find these arguments hard to accept.

Porter[8] suggested that research scientists should follow Schweitzer's principle of 'respect for all life' and become anti-vivisectionists at heart. This apparent contradiction would act as an internal regulator of experimental design under the guidance of ethical tool kits, one of which he proposed. The three Rs of reduction, refinement and replacement have grown to become a popular shorthand against which to judge ethical tool kits, and are discussed in more detail below.

One consequence of the need for experimental planning is the creation of a convenient framework for assessment of likely harms and potential benefits. This has been seized upon by all sides of the debate, apart from abolitionists, as a means to judge the merits of any given proposal. The resulting mechanisms for practical ethical appraisal are more advanced than in most other spheres of veterinary work. For example, the ethical framework around using prosthetic limb replacements for companion animals is in an early stage of construction[9].

The majority opinion today is a reluctant acceptance of the need to use animals in certain circumstances (although these differ markedly between individuals), and a wish for independent regulation such that scientific users of animals are answerable to society at large. The argument turns on whether society is prepared to set aside current and future benefits that may arise from animal research on the basis of a moral obligation to the species used. The philosophy that best describes this balance of public sentiment is utilitarianism. Crucially, utilitarianism does not rule out any harms as a matter of principle. However, parts of the regulatory framework of both the UK and EU override utilitarianism in an attempt to mirror majority public opinion of ethical 'lines in the sand'. These elements arise from the philosophy of deontology which argues that some actions should be seen as moral duties to be adhered to whatever the consequences. A problem with mixing these two approaches is knowing when to apply each principle and in particular how to manage cases on the margins. This may be illustrated by the following examples.

- At what point in the phylogenetic tree should animals fall under ethical considerations and legal protection? EU Directive 2010/63, reflected in UK law, protects all living vertebrates and cephalopods. This suggests that these species matter whereas the remainder do not. Other species considered for inclusion during drafting of the Directive included decapod crustaceans, some spiders and insects such as honey bees. Complexity (number of synapses) of the brain is not always a helpful measure of sentience or suffering[10] and the evolutionary tree has many examples of convergent as well as divergent evolution. Natural selection produces species that are best fitted to their environment, not those that are the most sentient. The idea that there is a link between degree of evolutionary development and capacity to suffer is a gross over-simplification, but is used as a basis for regulation. A purely ethical approach to regulation might be to confer the benefit of the doubt for any species where sentience is disputed.
- During the drafting of EU Directive 2010/63 calls were made for a ban on experiments involving severe or prolonged suffering, irrespective of the perceived need or justification. In effect, the harm/benefit balance would have deontological limits applied. Problems with this approach include how to define 'severe and prolonged suffering' and how to act in the unlikely event of overriding need, such as a lethal human pandemic. In Directive Article 15 it states that 'a procedure is not performed if it involves severe pain, suffering or distress that is likely to be long-lasting and cannot be ameliorated' but again this is subject to the *force majeure* arguments of Article 55 which allows the 'line in the sand' to be circumvented.
- Selling cosmetics that have been tested on animals was banned throughout the EU in 2009. The reduction in the use of animals for this purpose has been widely welcomed. However, a difficulty arises on the margins of defining a cosmetic. Substances such as botulinum toxin have a licence for only medical use but are also used for primarily cosmetic purposes; batches have to be tested for potency using animals, regardless of their end use. Should sunblocks, which reduce the risk of skin melanomas, be classed as cosmetics or medicines?
- Special protections are offered under UK law for research dogs, cats, equids and non-human primates. The reason is more to do with the special emotional attachment that humans have for these species than any scientific grounds. No special

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protections are offered for species with probably overlapping degrees of sentience such as pigs, sheep, dolphins, foxes or even crows. Should not all sentient species be protected equally?

Once deontological exclusions have been applied, the remaining uses of laboratory animals are usually judged according to principles of utilitarianism, a development of consequentialist reasoning. Consequentialism argues that the value of an action is determined by its outcome. To go forward, the harms and benefits of any action need to be known and compared and the balance of benefit over harm should be greater than any other feasible option. Utilitarianism, espoused by philosophers such as Bentham, further argues that there is a moral duty to maximise the balance of benefits to harms7. Within laboratory animal use this has become the cornerstone of ethical judgements. The system has the perceived advantage of each proposal receiving case-by-case attention with no preconceptions.

An objective harm/benefit analysis must multiply the value of a hoped-for benefit by the likelihood of achieving it, before weighing that estimate against the predictable harms. However, a major problem for any utilitarian analysis is that the (anticipated) benefits and harms are measured in different units; the old problem of comparing apples and oranges. This is part of the problem of seeking to make ethics a matter of quantification. In the light of this difficulty the ethical review process in the UK requires the presence of a number of experts plus lay members to provide balance and increase public confidence[11]. This variety of experts can help a committee to appreciate the variety of harms and benefits involved, and offset any crude attempt at precise quantification. Potential benefits are easy to over-estimate. It is difficult for proponents of a particular project to remain objective, particularly when career progression is involved. Harms may be easier to predict but are difficult to measure. Limiting harms effectively is directly dependent on local standards of animal care put in place by management and the competence of staff to adhere to those standards.

The Local Ethical Review Process

Since 1 April 1999 all designated establishments have been required to implement a local ethical review process, or ERP. Under the UK Animals (Scientific Procedures) Act an establishment is required to have an Animal Welfare and Ethical Review Body. The aims of the process are to provide independent ethical advice to the holder of the Section 2C licence, to provide support to the animal care staff and designated veterinary surgeon and to use ethical analysis to increase awareness of animal welfare issues and develop initiatives to lead to the widest possible application of the three Rs. This makes sure that all use of animals at the establishment is carefully considered, that all possibilities for reduction, refinement or replacement are taken into account and that high standards of accommodation and care are achieved. The structure of the ERP should be designed to suit the size and nature of the establishment, and may consist of committees, e-mail discussion groups, project-refinement groups or other formats, all of which should encourage local consideration of ethical issues. In general, as many people as possible should be involved in the ERP, to widen the debate as much as possible. The animal care staff, designated veterinary surgeon and representatives of the users should also be included. The ERP should include people who do not use animals, and lay people from outside the institution may also be appropriate members. The local ERP provides a mechanism to help holders of a Section 2C licence to meet their responsibilities and encourage wider local involvement in addressing issues surrounding animal use. The inclusion of lay people from the local community among the membership when debating the issues may go some way toward satisfying the demand for public accountability of what research should be allowed to be performed on animals in the name of the public interest. The holder of the Section 2C licence has to decide not whether the proposed work *can* be done, but whether it *should* be done.

Measuring harms and benefits

Measuring benefits

Predicting beneficial outcomes of experiments before they have been conducted is fraught with difficulty. If outcomes were known in advance then experiments would be redundant. The nature of scientific enquiry depends more upon testing the reliability (or falsifiability) of a hypothesis than proving unequivocal truths[12]. However, scientific history is full of instances when progress in fundamental knowledge, using animals, has been shown after the event to have been crucial for advances in medicine and human quality of life. Treatments for life-threatening diseases such as polio, diabetes and smallpox are often cited. Some of this progress might have been achievable by other means, but we cannot know this.

The funding for research using laboratory animals arises from three main sources: government grants (national or international), charities and the biomedical and chemical industries. The first two categories usually require peer-reviewed grant applications, a

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process which examines claims of potential benefits. However, industry-driven research ultimately requires a return on investment, which to some extent skews the selection of research area as well as placing demands on efficiency.

Measuring harms

The modern concept of welfare, which is now promoted by the Farm Animal Welfare Council[13], is that of a life worth living, from the animal's point of view, or, even better, a good life. A judgement of the quality of life of experimental animals against the benefits to other animals, or humans, is the basis of the harm/benefit analysis. It is much more than simply the prevention of cruelty: quality of life should be used for decision-making and to do this a quality-of-life balance sheet can be prepared[14]. Proper welfare assessment must be carried out. For more detail on this, see Chapter 4.

Individual measures of welfare are most valuable when considered in conjunction with other welfare-related parameters. The welfare assessment matrix[15,16] combines the clinical condition of the animal, behavioural deviations, the duration of the incident and the quality of the environment and shows how a grid can illustrate the temporal component of suffering. Since it is cumulative suffering that matters to the animal's quality of life, as much as single incidents, the points of suffering can be added up over a period of time to determine when a limit has been reached. The matrix includes contingent as well as direct suffering to truly reflect cumulative harms and allows an evaluation of the animal's quality of life. It can demonstrate the true welfare implications of research and the effect of refinements, both at the planning stage and when reviewing finished work, to ensure that harm/benefit scoring remains valid, and by the inclusion of a factor that reflects the justification can provide a weighting for the cumulative impact of the suffering incurred.

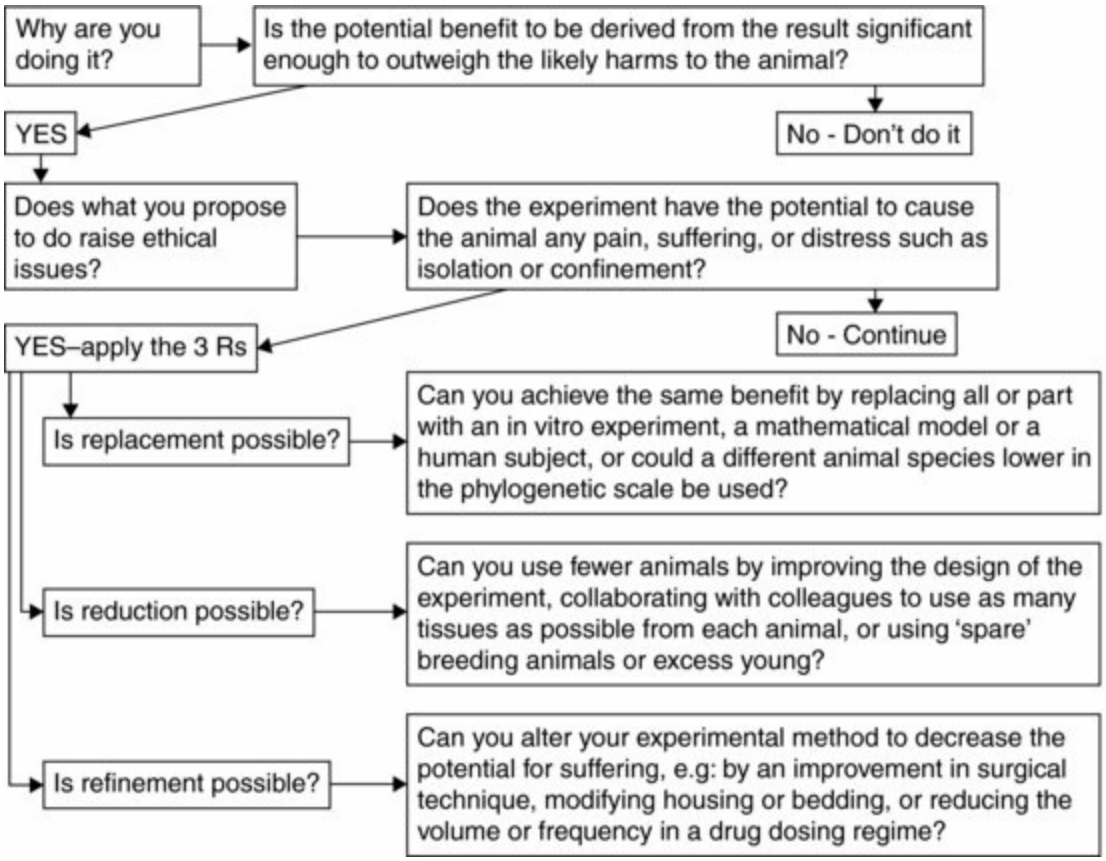
The Principles of the Three RS

The successful application of the three Rs has improved the use of laboratory animals and in some areas enabled progress without any animal use at all. EU Directive 2010/63 makes explicit reference to the three Rs and requires that ‘member States should contribute through research and by other means to the development and validation of alternative approaches’.

Regulated procedures using animals involves inflicting potential harm on them. The motive for this is usually to derive some benefit to humans or other animals. For research to be conducted in an ethical manner the researcher should analyse the motives behind the research: is it being done for a good enough reason, and could it be done any other way? Therefore before embarking on an experiment involving animals it is important to consider the questions in the flow chart in Figure 2.1, to apply the three Rs, and at all times throughout the experiment, consider what could be done to decrease the potential for suffering inflicted on the animals, and to maximise the likely benefit to be derived.

FIGURE 2.1 Application of the three Rs.

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Replacement

Alternatives are often argued not to be 'the real thing', but neither are live animal models. In the field of toxicity testing, the US National Academy of Sciences[17] has put forward a vision of replacement using high-throughput *in vitro* screening assays, tests in 'lower' organisms, systems biology, functional genomics and transcriptomics as well as predictive *in silico* approaches. Practical moves towards replacement among regulatory authorities include the Organisation for Economic Co-operation and Development (OECD) guidelines[18] accepting the use of artificial human skin as a replacement for living skin in the testing of chemical irritancy. Groups such as the European Centre for the Validation of Alternative Methods (http://ecvam.jrc.it/), the European Partnership for Alternative Approaches (http://ec.europa.eu/enterprise/epaa/), the Centre for Alternatives to Animal Testing – Europe (http://caat.jhsph.edu/about/_includes/index_eu.html) and the In Vitro Testing Industrial Platform (www.ivtip.org/) have risen to prominence in recent years as impetus (and funding) has increased the rate of discovery and application of alternatives to chemical and drug safety testing.

Reduction

When animals cannot be replaced, proper planning of experiments is required in order to produce reliable data with the fewest and most suitable animals. The application of statistical methods such as power analysis and blocking, and the use of inbred strains of animal to reduce variation have been advocated for many years[19] with some success. Yet it remains frustrating that surveys of published data continue to show improper designs or a lack of uptake of these ideas[20].

Refinement

Refinement can improve welfare by acting on both direct and contingent suffering. The search for refinement must be continual to take advantage of technological advances.

Academic research tends to be very focused into narrow areas. Scientists are strongly driven to do high-quality research in

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their field, but may lack similar drive when evaluating the influence of methodology on the scientific quality of their results or on the welfare of animals used. The implementation of the three Rs is seen almost as a separate subject area, whereas it would be better if seen as a normal part of the research package and integrated into it. Within the pharmaceutical sector, strong corporate drivers for consistency and harmonisation within global organisations can be used to bring about refinement (e.g. housing improvements) and in an increasingly tough external environment, a strong focus on corporate reputation can also bring useful leverage to welfare initiatives.

Some current examples of refinement include

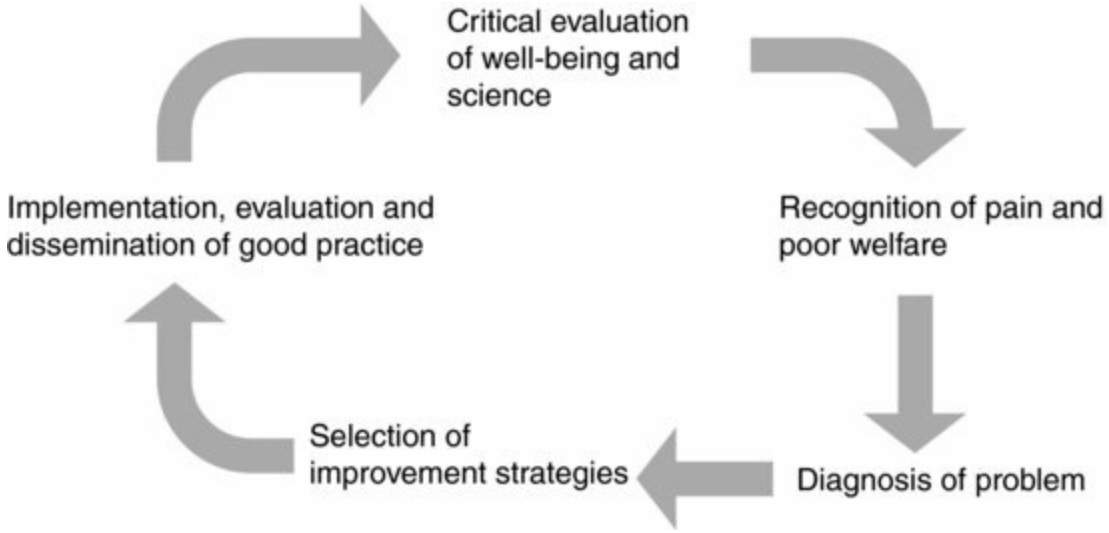
- the almost universal application and continuous updating of anaesthesia and analgesia methods,
- a refined non-surgical technique involving trans-cervical instillation of genetically altered embryos into the uterus of surrogate recipients, potentially rendering laparotomies redundant for many thousands of mice[21],
- clear evidence that the usual method of capturing and picking up mice (by the tail), results in anxiety compared with cupping, and may impinge on welfare[22].

A key requirement for the three Rs is sharing accurate data. There remains a reluctance from scientists and publishers to use print space to report negative results or experimental 'failures', which, if overcome, would avoid needless repetition[23]. Sufficient detail on animal care for experiments to be reliably repeated without errors is often lacking[24]. The Animals in Research: Reporting In Vivo Experiments (ARRIVE) guidelines are a welcome initiative designed to correct this deficiency[25].

Prospective, ongoing and retrospective review: reflective practice and the refinement loop

Refinement is an iterative process, which begins with a critical evaluation of practice, leading to recognition and assessment of poor or suboptimal welfare, identification of the causes of this, selection of improvement strategies and implementation of these strategies. Any changes then have to be evaluated for their efficacy; thus the process begins again, forming the refinement loop (see Figure 2.2)[26].

FIGURE 2.2 The refinement loop26.



Critical evaluation of practice

Researchers have to look critically at what they do and identify whether there is a better way of conducting the research that will minimise welfare implications and maximise the scientific output, thus improving the harm/benefit balance and the justification for doing the work. It is particularly important that this is seen as an ongoing requirement: new developments in refinement may turn what was yesterday's best practice into today's outdated methodology.

Objective assessment of animal welfare and scientific quality

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This requires first and foremost an understanding of well-being and what particular animals need. Only then is it possible to identify appropriate parameters to assess well-being and scientific quality in a meaningful manner. Sufficient resources have to be provided to perform the assessments effectively. This requires the researcher to schedule observations appropriately to maximise the likely detection of problems, to recognise where there is deviation from normal, taking into account species, strain and inter-animal differences and to be able to identify when there is room for improvement.

Evaluation of improvement strategies

Having implemented an improvement strategy it must be evaluated to determine whether there has been an improvement. It may be that the new method is found to be better, or that the current method is confirmed as being the best one available; either way, the information is valuable to others in the field. This information should then be disseminated to others working in the same area, ideally by publication in mainstream journals, but as a minimum it should be used for internal reference.

It is essential to coordinate the approach to refinement within an institution or organisation. It may be beneficial to identify someone with overall responsibility for this: a 'refinement champion' who is responsible for ensuring that refinements are actively implemented at the establishment. The institution needs to ensure that support is available for key people in developing a culture of care, and that the professional integrity of the experts in welfare assessment is recognised. The animal management team needs to communicate effectively with senior management, to make sure that adequate resources are provided to implement refinements appropriately. There should be a clear process for the implementation and reporting of problems, and the development of a culture of advice-seeking and support, not blame and isolation. Scientists need to recognise that there is a learning curve to all new methods, and accept that while inexperience may lead to new or increased problems in the short term, there will be longer-term benefits from perseverance. Refinements may be developed to enhance animal welfare, to improve scientific quality or sometimes simply as a defensive reaction to prevent criticism of old-fashioned methodologies. The underlying reason of why a procedure is done matters less than what is done, when, how and by whom.

Public Perceptions

The polling company Ipsos MORI has been asking the public in the UK about their views on animal research since 1999[27]. Among their findings, as reported by the organisation Understanding Animal Research (www.understandinganimalresearch.org.uk/your_views), was that throughout this time more than 80% of respondents accepted the need for animal research provided that certain conditions were being met:

- that there is no unnecessary suffering,
- that the research is for serious medical or life-saving purposes,
- that there is no alternative.

Much of the existing regulatory process is built around a desire to satisfy these caveats. The Ipsos MORI poll showed that, since 2005, a majority of the surveyed population 'expect that the rules in Britain on animal experimentation are well enforced'. A significant, and often vocal, minority of the population are opposed to any use of animals in research and campaigning organisations such as the British Union for the Abolition of Vivisection (BUAV) argue that 'harming animals in the name of science is morally indefensible' (www.buav.org/humane-science).

For the most part, society accepts that the use of animals is necessary for medical progress, provided there are assurances over the purpose for which the work is carried out and the degree of suffering inflicted. Surveys have shown that people will accept a greater degree of suffering to animals in the pursuit of cures for devastating illnesses such as childhood leukaemia than for minor problems or for toxicity testing[28]. However, there is a significant sector in society which believes that however high the anticipated benefit no animal should undergo more than transient suffering, which may be at odds with the interests of science and industry. A majority of people seem to support the use of mice in fundamental biomedical research if they come to no harm, but begin to show disapproval as soon as pain, surgery or illness became involved. If animal suffering cannot be ruled out it may be hard to convince the public of the value of fundamental biological research. However, much research of this type is relatively benign, and so might win public support if measures to limit suffering were implemented effectively and described in detail. It is up to the scientist therefore to fully explain the nature of the work being undertaken, and to put it in context, such that the public can understand the motives for the work and gain a greater understanding of research.

In contrast to the many examples in which animals suffer pointlessly (it could be argued; such as over-exploitation of sporting animals in competitions, cosmetic mutilations or organised dog fighting) at the hands of people, usage of laboratory animals in

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experimental procedures is purposeful and is ethically scrutinised in advance. However, this process leads to the harms that might occur being premeditated, which may lead to accusations of scientists being unemotional, inhumane or even abusive[29]. Although animal research has historically received public criticism similar to activities such as hunting, fishing and factory farming, the moral case for carefully controlled research using animals is actually much more defensible. And yet, an individual animal's perception of pain and suffering is the same whatever the context or 'reason' for its life. A laboratory animal will not have the benefit of knowing that its harm is for a 'good cause'; nor can it give permission. A system to compare life experiences of laboratory animals with farm, companion or wild animals has been proposed15.

Transparency and freedom of information

Modern society demands accountability in laboratory animal use. If this use is truly defensible and ethically robust, it should be more transparent. Labelling drugs and household substances to show whether animals were used during development could be part of this. This would build trust, assist those who do not wish to use products derived from experiments involving animals and increase uptake of the three Rs. However, some patients may refuse important treatment. Much of the information held in research institutes and by the funding agencies is now available under the UK Freedom of Information Act. This has caused some concern as researchers worry about the security implications of actions by animal rights protesters.

Campaigners in the UK have historically played a vital role in bringing about legislation to control animal research. Martin's Act of 1822 against cattle cruelty, the Cruelty to Animals Act 1876 and the Animals (Scientific Procedures) Act 1986 all owed an enormous amount to campaigners bringing the issues into the public arena. Activism has directly influenced the research of some groups[30] and different parts of the scientific community have either adopted greater public openness or been driven to regrettable but understandable secrecy. Animal cruelty has no doubt occurred in the history of research and anti-vivisectionists have rightly called attention to it. Establishment in 1999 of the local ERP owes much to the importance placed on animal welfare and the ethics of animal research. There is now a degree of peer pressure in many establishments to ensure that the welfare of animals is paramount, both for its own sake and for the management of wider reputational risks. The modern research community generally puts the ethics of animal experimentation at the heart of all its work, and the aim for the future must be not only to maintain this high standard but to continue openly to seek improvement.

During a 2-day conference in Basel in 2010, more than 80 life science researchers from Switzerland, Germany, Sweden, France and Great Britain addressed the problems of animal research and adopted the Basel Declaration (www.basel-declaration.org/). The Declaration marks an unprecedented effort of the scientific community towards more trust, transparency and communication on animal research. The signatories commit to accepting greater responsibility in animal experiments and to intensive cooperation with the public, and also with national and international decision makers. They are actively seeking to show that science and animal welfare are not diametrically opposed and to make a constructive contribution to the dialogue taking place in society.

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Chapter 3

The Regulatory Framework

Introduction to Legislation Regulating the Use of Animals in Research

William Harvey is considered the inventor of modern laboratory science. He discovered the circulation of the blood, using both dead and live animals, and published his book *On the Motion of the Heart and Blood In Animals* in 1628[1]. Harvey's discovery of circulation led to extensive use of vivisection in Europe. In the 19th century, French physiologist Claude Bernard published *An Introduction to the Study of Experimental Medicine.* This book provided a sound rationale for scientific medicine, which greatly accelerated its development. By the early 20th century, the knowledge accruing from the increasing use of animals in scientific research was testimony to the success of the method.

The first anti-vivisection societies originated in 1875, and the best known of these was the Society for the Protection of Animals Liable to Vivisection, founded by Frances Power Cobbe, which later became the Victoria Street Society (VSS) and then the National Anti-Vivisection Society (NAVS). Miss Cobbe then left the NAVS, and founded the British Union for the Abolition of Vivisection (BUAV) in 1898. Both the NAVS and BUAV remain very active, campaigning in schools, via the media and in the political arena for a ban on the use of animals in research.

The VSS presented the first anti-vivisection bill in history to the House of Lords in 1875. The scientific community were obliged to present a bill themselves in opposition to this, and following these bills a Royal Commission was set up to study the issues. The result of this was the Cruelty to Animals Act 1876, the first law written specifically to regulate animal experimentation. This Act controlled experiments causing pain to living animals, and set up a system of licensing and certification. The central purpose of the law was to reconcile the needs of science with the just claims of humanity and this principle is implicit in all the various laws, conventions and directives which have been passed since.

In 1926, Charles Hume founded the University of London Animal Welfare Society (now the Universities Federation for Animal Welfare, UFAW). He published the first edition of the *UFAW Handbook on the Care and Management of Laboratory Animals* in 1947 (now in its eighth edition). He also commissioned Russell and Burch[2] to write *The Principles of Humane Experimental Technique* in 1959, which brought out the concept of the three Rs: Replacement, Reduction and Refinement.

The 1876 Act stood for 110 years. In the 1960s it became necessary to update the legislation. Pressure for change continued into the 1970s and in 1976, coinciding with Animal Welfare Year and the Centenary of the 1876 Act, the Committee for Reform of Animal Experimentation (CRAE) was formed. In 1983 CRAE, the British Veterinary Association (BVA) and the Fund for Replacement of Animals in Medical Experimentation (FRAME) joined forces to negotiate the White Papers, and the Animals (Scientific Procedures) Act (or ASPA) was passed in 1986.

European legislation

In 1985, the member states of the Council of Europe agreed a series of guiding principles for the protection of vertebrate animals in experimental or other scientific procedures. These principles were published as European Convention ETS 123[3]. This Convention set out minimum requirements for the care and use of animals in scientific procedures. Council of Europe conventions are not binding on member states unless a state ratifies it, and agrees to implement its provisions. The European Union ratified the Convention in 1998, therefore agreeing that all EU member states would implement its provisions in their national legislation. Minor changes were made to the Animals (Scientific Procedures) Act 1986 at this time to ensure that all provisions of the Convention were fully implemented in the UK.

Changes to the Convention require amendments to relevant national legislation. Changes to ETS 123, to reflect advances in knowledge, were agreed in 2006, and the EU published Directive 2010/63[4] in September 2010, informing EU member states of

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new minimum legislative requirements for the protection of animals in research. The deadline for implementation of these requirements was 1 January 2013, and the Animals (Scientific Procedures) Act was amended in 2012 to take account of these changes.

The Animals (Scientific Procedures) Act 1986

The Act implements the requirements of EU Directive 2010/63, and lays down rules in relation to the care and use of animals in research in order to minimise the severity experienced by the animals and safeguard their welfare. It aims to ensure that any work involving animals is original and justified, and that there is no non-animal alternative. It requires that organs and tissues of killed animals should be shared if possible, and that support should be given to develop and validate alternative strategies which do not use protected animals, or which use fewer animals, or reduce the pain, suffering or lasting harm caused to protected animals. In this way, the system of regulation aims to ensure compliance with the principles of the three Rs. Wherever possible, a scientifically satisfactory method which does not require the use of animals must be used. If animals do have to be used, the number used must be reduced to a minimum, and the breeding, accommodation and care of animals and the procedures used must be such as to eliminate or reduce to the minimum any possible pain, suffering, distress or lasting harm caused. In addition, the actual severity experienced by the animals used must be assessed and recorded.

The Act controls *regulated procedures* performed on *protected animals* (see Box 3.1), and does this by licensing at three levels, the person, the project, and the place.

Box 3.1 Summary of protected animals and regulated procedures

- A *protected animal* is defined as 'any living vertebrate, other than man, and any living cephalopod'.
- Animals are protected from defined stages of development: mammals, birds and reptiles from the last third of gestation or incubation, other forms from the point of independent feeding. Embryonic cephalopods are not protected.
- A *regulated procedure* is a procedure applied to a protected animal for a qualifying purpose which may have the effect of causing that animal pain, suffering, distress or lasting harm.

Protected animals

The Act provides protection for certain types of animal used in scientific research. These are known as protected animals. A protected animal is defined as any living vertebrate, other than man and any living cephalopod.

Fetal, larval and embryonic forms are protected from certain stages of development. Mammals, birds or reptiles are protected from two-thirds through gestation or incubation. Fish and amphibia are protected from the point at which they become capable of independent feeding. Cephalopods are protected after they have emerged from the embryonic stage, usually at hatching.

Death is defined as the point at which there is permanent cessation of circulation or destruction of the brain.

Regulated procedures

A regulated procedure is one applied to a *protected animal* for an experimental or other *scientific purpose* (see qualifying purpose, below) which may cause the animal *pain, suffering, distress or lasting harm* equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice. All three conditions (in italics) must be fulfilled for the procedure to be regulated.

Procedures may also be regulated if they are part of a series or combination of procedures applied to the same animal for a qualifying purpose which together may cause pain, suffering, distress or lasting harm, even if they are innocuous individually, provided the animal is a protected animal throughout, or if in the course of the procedure it reaches the stage of development when it becomes one.

Procedures applied to animals before they become protected will also be regulated if the animal lives beyond the point at which it becomes protected.

Anything which may result in the birth or hatching of a protected animal is also a regulated procedure if it may cause pain suffering distress or lasting harm. This includes techniques which result in the modification of an animal's genes, such as

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pronuclear injections. In addition, the breeding of a genetically modified animal is a regulated procedure if the animal is allowed to live beyond the stage when it becomes a protected animal, and it may experience pain, suffering, distress or lasting harm as a result of the genetic modification.

The use of anaesthesia or analgesia is disregarded when making the judgement about whether there may be any harm; hence procedures carried out on anaesthetised or decerebrated animals are still regulated under the Act.

Exemptions

Certain procedures are exempted from regulation under the Act. Ringing, tagging or marking an animal primarily for identification is not regulated, provided it causes no more than momentary or transient pain and no lasting harm. Examples of appropriate methods are ear tagging, tattooing or microchips.

Recognised veterinary, agricultural or husbandry practices are also not regulated under the Act, although these may be covered by other legislation (see Table 3.2, below). Similarly, administering substances as part of a veterinary clinical trial is not regulated under the ASPA.

The humane killing of a protected animal is a regulated procedure only if the animal is killed for an experimental or other scientific purpose, the place where it is killed is licensed under Section 2C of the Act or is a place specified in a project licence, and the method employed is *not* as described in Schedule 1 to the Act or specified on the relevant 2C licence. Schedule 1 is a list of approved methods of humane killing that do not require licence authority. See Chapter 8 on euthanasia for details.

Licensing of places

Section 5 of the ASPA requires that places specified in a project licence must be licensed under Section 2C, unless it appears necessary to specify a different place, for example if procedures are to be carried out in the wild or on farms. Most often, regulated procedures are therefore performed at licensed scientific procedure establishments. Establishments where particular types of animal are bred or kept for supplying to research establishments, for either *in vivo* or *ex vivo* use, must also be licensed under Section 2C. These types of animal are specified in Schedule 2 to the Act (see Table 3.1).

Table 3.1 Animals included in Schedule 2 to the Animals (Scientific Procedures) Act.

Mouse (*Mus musculus*)
Rat (*Rattus norvegicus*)
Guinea pig
Hamster (Syrian, *Mesocricetus auratus*, and Chinese, *Cricetulus griseus*)
Rabbit (*Oryctolagus cuniculus*)
Dog
Cat
Primate
Any bird of the species *Coturnix coturnix* (quail)
Ferrets
Gerbil (*Meriones unguiculatus*)
Frogs of the species *Xenopus laevis*, *Xenopus tropicalis*, *Rana temporaria* or *Rana pipiens*
Zebrafish

Licences under Section 2C are only issued if the Secretary of State at the Home Office is satisfied that the prospective holder and the place itself comply with the requirements of EU Directive 2010/63.

The application for a 2C licence must identify:

1. a person responsible for overseeing the welfare and care of the animals kept (named animal care and welfare officer, NACWO);

2. a veterinary surgeon, or other suitably qualified person, to provide advice on the health and welfare of the animals (named veterinary surgeon, NVS);

3. a person responsible for ensuring that the persons dealing with animals have access to any information they need about the species concerned;

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Box 3.2 The system of control

Regulated procedures may only be carried out if:

- performed by the holder of a *personal licence*,
- authorised by a *project licence*, and
- the *place* is *specified in the project licence.*

4. a person responsible for ensuring that the persons working with animals are adequately educated and trained and are supervised until they have demonstrated competence (Training and Competency Officer, TCO); and

5. a person responsible for ensuring that the conditions of the licence are complied with.

Note that the same person may be nominated for two roles. If it appears to either the person responsible for animal care or the veterinary surgeon that the health or welfare of an animal gives rise to concern, that person must either notify the relevant personal licence holder or take steps to ensure that the animal is cared for or killed if necessary, using a Schedule 1 or other appropriate method. Thus the NVS and NACWO have a responsibility in addition to that of the research personnel to safeguard the welfare of animals used.

Section 3 of the ASPA outlines the system of control (see Box 3.2). It states that 'no person shall personally apply a regulated procedure to an animal unless:

a. he holds a *personal* licence qualifying him to apply a regulated procedure of that description to an animal of that description;

b. the procedure is applied as part of a program of work specified in a *project* licence authorising the application, as part of that program, of a regulated procedure of that description to an animal of that description; and

c. the *place* where the procedure is carried out is specified in the project licence.'

Thus, three types of licence are required to be in place before a scientific procedure may be carried out on an animal.

The personal licence

The personal licence qualifies the holder personally to apply regulated procedures of specified descriptions to animals of specified descriptions. The species to be used are divided into groups for this purpose, and procedures are assigned a category, depending on the skill level required to conduct the procedure and whether recovery anaesthesia is used (see Box 3.3). Proposed species groups and categories are outlined in Box 3.3. Note that this may be subject to change and it is advised that the Home Office Inspector be consulted for definitive information.

Before applying for a personal licence, the applicant must usually have completed training appropriate to the species to be used and category of procedures to be included in the personal licence application, and provide evidence of this training when submitting the application. The content of such appropriate training courses is outlined in Chapter 1. The training and competency officer at the institution will be responsible for ensuring that the training is undertaken, and subsequent to obtaining a licence that the person is appropriately supervised, and develops competence in the techniques.

Box 3.3 Proposed species groups and categories of techniques for personal licence applications

Species groups

- Rodents (including mice, rats, guinea pigs, hamsters and gerbils) and rabbits
- Small carnivores (including dogs, cats and ferrets)
- Non-human primates (Old World, e.g. macaques, and New World, e.g. marmosets)
- Farm animals (including pigs, goats, sheep and cattle)
- Horses
- Birds
- Reptiles
- Amphibians
- Fish
- Cephalopods

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Categories of procedure

A. Procedures not requiring any anaesthesia

B. Procedures requiring simple anaesthesia for restraint or non-recovery

C. Procedures requiring more complex anaesthesia plus surgery

D. Procedures requiring anaesthesia plus administration of neuromuscular blocking agents

Applications for personal licences are usually endorsed by the training and competency officer nominated in the Section 2C establishment licence, who has knowledge of the education, training, experience and character of the applicant.

Personal licences are only granted to people over the age of 18 with appropriate education and training, who are competent to apply those procedures and to handle and take care of laboratory animals. Personal licences are reviewed by the Secretary of State every 5 years.

A personal licence can be used under any valid project licence, but can *only* be used in conjunction with a project licence: it does not authorise the performance of any procedures alone. The licence includes details of the groups of animals on which procedures may be carried out, the categories of technique that may be applied and a set of conditions defining the responsibilities of the personal licence holder.

The personal licence holder bears primary responsibility for the health and welfare of animals on which they have carried out scientific procedures. The Guidance on the Operation of the Animals (Scientific Procedures)[5] Act 1986 must be read before applying for a licence.

Responsibilities of personal licence holders

Personal licence holders bear primary responsibility for the welfare of animals on which they have performed scientific procedures. They must ensure that they have the necessary authority on personal and project licences, and avoid unauthorised re-use or release of animals. They must label cages or pens appropriately and keep records of the procedures they have carried out, indicate whether they were supervised or not, and whether their supervisor deemed them competent. They must check their animals daily and make suitable arrangements for any period of absence. They must be familiar with the severity limits and the constraints upon adverse effects contained within the project licence, and seek veterinary advice and treatment if required.

The conditions attached to a personal licence further define specific responsibilities of the holder. These conditions require the holder to implement the three Rs as much as possible, minimising the number of animals used and reducing the duration and intensity of suffering caused, while using procedures that are most likely to provide satisfactory results. The procedures must also result in the death of as few protected animals as possible.

Personal licence holders must take precautions to prevent or reduce to the minimum any pain, suffering, distress or discomfort caused, consistent with the purposes of the procedure. If a procedure is seen to be causing severe pain, suffering or distress, steps must be taken to ameliorate these adverse effects, and any unnecessary pain, suffering, distress or lasting harm must be stopped. Death as an end point must be avoided as far as possible, and be replaced by an early and humane end point. No procedures may be applied that may cause the animal severe pain, suffering or distress which is likely to be long-lasting and cannot be ameliorated. If an animal is found to be in severe pain, suffering or distress that is likely to be long lasting and cannot be ameliorated, it must be killed immediately using an appropriate method. This applies regardless of how important the animal is for the experiment. The welfare of the animal comes first.

Anaesthesia (general or local) should always be used, unless:

- analgesia (or another appropriate method) is used to ensure that the adverse effects caused by the procedure are kept to a minimum;
- the procedure will not cause severe pain; and
- the use of anaesthesia would be more traumatic to the animal than the procedure itself or would frustrate the purposes of the procedure.

In any case, analgesics or other pain-relieving methods must be used to ensure that animals do not experience any pain once the anaesthesia wears off.

Competence and supervision

Personal licence holders must achieve and maintain competence in the procedures used. Until technical proficiency and competence are attained, the personal licence holder must be supervised appropriately. The level and extent of necessary

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supervision and training should be reviewed and agreed periodically. Initially, direct supervision may be required, but as technical competence is gained the level of supervision can be reduced. However, it is important for new researchers to continue to seek guidance even once competence is achieved. Supervision does not lessen the individual responsibility of the personal licensee to comply with the provisions of the Act and the terms and conditions of the licence.

Delegation of authorities

A personal licence holder may request authority from the Secretary of State to use unlicensed assistants to perform certain techniques specified on their personal licences that require no technical skill or knowledge, such as the filling of food hoppers and water bottles with previously altered diets or liquids, or pairing/grouping associated with the breeding of animals. The personal licence holder remains responsible for the welfare of the animals involved.

The competence of the personal licence holder is a key factor in maintaining the welfare of laboratory animals. Personal licence holders should take responsibility for ensuring that they are fully trained and competent before performing regulated procedures, and for seeking advice and assistance when necessary.

Project licences

A project licence sets out a programme of work, and authorises the application, as part of that programme, of specified regulated procedures to animals of specified descriptions at specified places. A 'programme of work' is considered to be the combination of regulated procedures and end points to be applied to protected animals to achieve the scientific objectives specified in the project licence. Section 5 of the ASPA sets out the requirements of the Act for project licences.

The project licence details the background to and the likely benefits from the specific work to be carried out and the likely harm to be caused to the animals. The plan of work indicates how the purposes are to be achieved, linking the performance of specified regulated procedures to the achievement of the purposes. Protocol sheets describe the procedures to be performed on individual animals, including details of the likely adverse effects of the procedures, and what steps will be taken to minimise these, including the use of analgesics and humane end points. A severity classification is stated for each individual protocol: these may be mild, moderate, severe or unclassified, which is used if the procedure is conducted entirely under terminal anaesthesia. The severity categories are not discrete entities but form a spectrum across a wide range of adverse effects. It is necessary to consider not only the immediate effects on the animal, but the longer-term effects as well, to gauge the overall effects of the procedure. Also, if corrective action is taken to prevent suffering at an early stage, the severity level may be kept down. Thus, the Act requires that the likely benefits are maximised, while harm caused to the animals is minimised.

Project licences are issued to someone who can take overall responsibility for the work. The holder should have expertise in the particular area of science, and will generally need to be proficient in the regulated procedures to be performed. They should also be able to design and manage experiments (including expertise in statistics where appropriate), supervise and guide personal licensees, and implement the three Rs of Reduction, Refinement and Replacement as much as possible. Therefore they must been trained in a relevant scientific discipline, demonstrate that they have specific knowledge relating to the species of animal to be used and have received training in designing programmes of work.

Assessment of projects and severity classification

Project licence applications are assessed before granting, to ensure that:

- the programme of work is justified from a scientific or educational point of view or is required by law;
- the purposes of the programme of work justify the use of protected animals; and
- the regulated procedures will be applied in the most humane and environmentally sensitive manner possible

The Secretary of State will evaluate the objectives of the programme of work and its predicted scientific benefits or educational value, and assess the compliance of the programme of work with the principles of Replacement, Reduction and Refinement. He will also assess the likely severity of the procedures to be used and assign them a severity category, be it *non-recovery*, *mild*, *moderate* or *severe*. A harm/benefit analysis will then be carried out to assess whether the harm that may be caused is justified by the expected benefit to man, animals or the environment. The Secretary of State will also determine whether the programme should be assessed retrospectively. This will usually be the case if the project involves non-human primates or severe procedures. A retrospective assessment will assess whether the programme of work has been carried out, whether the objectives were achieved and the amount of harm actually caused to the animals. It will also determine whether any lessons can be learnt from the programme of work that may contribute to the further implementation of the three Rs.

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Qualifying purposes

The ASPA restricts the purposes for which licences may be issued. These are known as qualifying purposes, and are:

- basic research;
- translational or applied research with one of the following aims:
  - the avoidance, prevention, diagnosis or treatment of disease, ill health or other abnormality, or their effects in man, animals or plants,
  - the assessment, detection, regulation or modification of physiological conditions in man, animals or plants, or
  - the improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes;
- the development, manufacture or testing of the quality, effectiveness and safety of drugs and foodstuffs;
- the protection of the natural environment in the interests of the health or welfare of man or animals;
- research aimed at preserving the particular species of animal;
- higher education or training for the acquisition, maintenance or improvement of vocational skills;
- forensic inquiries.

Project licences may not be granted for programmes of work which fall outside these categories. Project licences are valid for a maximum of 5 years, after which time a new application must be made if the programme of work is to continue. Non-technical summaries must be submitted with a project licence application, for publication.

Project licence conditions

Project licences are subject to a number of conditions. Like personal licence conditions, they require that the principles of Replacement, Reduction and Refinement are complied with. The procedures must use the minimum number of animals with the lowest capacity to experience pain, suffering, distress or lasting harm; cause the least pain, suffering, distress or lasting harm; and be most likely to provide satisfactory results. Procedures must not be applied if the procedure may cause severe pain, suffering or distress that is likely to be long-lasting and cannot be ameliorated. Procedures must result in the death of as few protected animals as possible; and the duration and intensity of suffering caused to those animals that die must be minimised. In any case, once the purpose of the procedure has been achieved, the procedure must be stopped and appropriate action taken to minimise the suffering of the animal. A decision must be taken by a veterinary surgeon or other competent person at the end of the procedures as to whether the animal should be kept alive or killed. If it is suffering or likely to suffer adverse effects from the procedure, the animal must be killed.

Additional conditions must be met before a project licence authorising the use of endangered animals, primates, cats, dogs or equidae will be granted, and no project licence will be granted authorising the use of great apes. Animals taken from the wild and endangered species are also afforded additional protection.

The project licence holder (also known as the PPLh) must ensure that personal licence holders working on the project are supervised until they have demonstrated the requisite competence.

To enable a project licence holder to manage a project efficiently everyone involved must be fully aware of the contents of a project licence and of their responsibilities. It is particularly important that everyone involved has access to an up-to-date copy of the licence.

Fate of animals at the end of procedures

The ASPA requires that once a series of regulated procedures is finished, the personal licence holder must ensure that the animals are killed if they are suffering or likely to suffer adverse effects. If a veterinary surgeon determines that they are not suffering or likely to suffer, then they may remain alive at the licensed establishment. Animals can then be set free or rehomed – for example to a farm or to be kept as pets – if certain conditions are met. Permission is required from the Secretary of State, the animal must be fit to be released, it must pose no danger if released, and measures must be taken to safeguard its well-being. For wild animals there are additional conditions. The fate of the animals should be specified on the project licence.

The Section 2C licence

The holder of this licence has overall legal responsibility for all the animal facilities and for all the procedures carried out in an institute. They have a number of responsibilities, including the following.

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- They must ensure that Replacement, Reduction and Refinement are implemented fully.
- A register of people must be maintained who are competent to kill protected animals, listing methods the person has been deemed competent to apply and on which species. People on the register must be trained and supervised until competent. There must be sufficient people trained so that someone is available at all times if an animal needs to be killed in an emergency.
- The installations and equipment at the establishment must be maintained such that they are appropriate for the species held and the procedures performed. This must permit reliable results to be obtained while using the minimum number of animals in procedures of minimum severity.
- The facilities must be maintained in accordance with the requirements of Annex 3 of EU Directive 2010/634. The fabric of the buildings in which experimental or breeding animals are kept must be maintained to a satisfactory standard, and the accommodation must provide adequately for the animals' needs, including quarantine and acclimatisation. The Home Office has issued a Code of Practice, based on the requirements in the previous EU Directive, in which the optimum environmental conditions and minimum cage sizes for protected animals were laid down[6]. This will be updated with the new requirements, which establishments will have to meet by 2017.
- They must ensure that sufficient staff are available to care for the protected animals kept. The staff must be adequately educated and trained, and supervised until they are competent.
- They must ensure that the physical environment and the well-being and state of health of animals are checked at least daily by a competent person, so that problems may be rapidly identified. All animals must be provided at all times with adequate care and accommodation appropriate to their type or species, and any restrictions on the extent to which the animal can satisfy its physiological and ethological needs must be kept to the absolute minimum.
- They must maintain an Animal Welfare and Ethical Review Body. This comprises as a minimum the person responsible for day-to-day care of animals and the veterinary surgeon. At an establishment where regulated procedures take place there must also be a scientist. This body provides advice on animal welfare; for example acquisition, accommodation, care and use of animals, the three Rs and advances in techniques. It may review internal processes and projects, including making assessments of adverse effects and implementation of the three Rs. The Animal Welfare and Ethical Review Body must keep records of advice given and decisions made.
- Records must be kept of the number and the species of animals bred, acquired, supplied, used in procedures, set free or re-homed.
- They must prevent unauthorised procedures. The Section 2C licence holder must ensure that all procedures carried out in the establishment are properly authorised, and must put in place systems of management that ensure compliance with the requirements of the Act and the terms and conditions of the licences.
- Identification of animals: dogs, cats and primates kept at the establishment must be individually and permanently identified and have an individual history file, which stays with the animal if it is moved. Equidae, farm animals and adult birds should also be identified. Each cage or pen should be properly labelled with the identity of the animals within.

Other responsibilities that the Section 2C licence holder is required to discharge include ensuring that health records are maintained under the supervision of a veterinary surgeon for animals held at the establishment, and ensuring that animals kept alive at the completion of regulated procedures are kept under the supervision of a veterinary surgeon unless moved, rehomed or re-used.

The named animal care and welfare officer

The Section 2C licence names a person responsible for the day-to-day care of the protected animals kept there (the named animal care and welfare officer or NACWO). This person has responsibilities laid down in the Act (statutory responsibilities), and other additional responsibilities. Their statutory responsibilities require that the NACWO should notify the relevant personal licence holder or arrange for the care or destruction of an animal if the health or welfare of that animal gives rise to concern. They may also notify the veterinary surgeon in such circumstances. The NACWO's responsibilities include both stock and experimental animals.

The NACWO is usually a senior animal technician, with expert knowledge and experience of animal technology. They ensure that husbandry and care of animals are carried out to the standards described in the relevant Codes of Practice. They will be familiar with Schedule 1 and other appropriate methods of killing. The NACWO will usually ensure that all the animals are checked daily by a competent person and that the environmental conditions are recorded. The NACWO should be familiar with the project licences in use and be involved in the local animal welfare and ethical review body. The NACWO and animal technicians will often be proficient in the techniques to be used and will be a valuable source of advice.

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The named veterinary surgeon

The Section 2C licence also names a veterinary surgeon to advise on the health and welfare of the animals (the named veterinary surgeon). The veterinary surgeon also has statutory responsibilities under the Act, and other additional responsibilities required by the Royal College of Veterinary Surgeons and Home Office. Named veterinary surgeons must undertake specific training, and there is an ongoing requirement for continuing professional development.

The statutory responsibilities require that the vet should provide advice on the health and welfare of the animals, and notify the relevant personal licence holder or arrange for the care or destruction of an animal if the health or welfare of that animal gives rise to concern.

In addition, the vet will visit all parts of the establishment frequently enough to be able to monitor the health and welfare of animals kept at the establishment. They are responsible for the diagnosis and treatment of diseases in the protected animals, and for directing the use of controlled and prescription-only medicines in the animals. They can advise on health screening and husbandry requirements, and will maintain health records. They will also be familiar with Schedule 1 methods of humane killing. The vet should be familiar with the project licences in use, and be able to provide advice to licensees regarding refinement of techniques, non-animal alternatives and selection of animal models. The vet may also perform techniques, training of licensees and the drafting of new applications and amendments. They should take an active role in the Animal Welfare and Ethical Review Body. Appropriate veterinary cover will be available at all times. The vet is a valuable source of advice and assistance, and personal licence holders are encouraged to seek advice from the vet and the NACWO on a regular basis.

Additional controls

Re-use

Section 14 describes the regulations relating to the re-use of animals, limiting the cumulative severity which any individual animal might experience. Once a series of regulated procedures for a particular purpose has been concluded, no animal may be re-used unless authorised by the Secretary of State. Animals may be re-used only if the actual severity of no more than one of the preceding procedures was classified as severe, and a veterinary surgeon with knowledge of the lifetime experience of the animal has advised that the animal's general state of health and well-being has been fully restored. The likely severity of the further procedure to be applied must be classified as non-recovery, mild or moderate. If the animal has experienced severe adverse effects, re-use will only be permitted where there is particularly strong justification.

Neuromuscular blocking agents

Neuromuscular blocking agents prevent an animal from moving, but do not diminish pain sensation. If used without appropriate analgesia or anaesthesia, there is the potential for pain and distress to go undetected. The use of these agents must be expressly authorised by the personal licence and the project licence, and they must be used in combination with an appropriate level of anaesthesia or analgesia. In addition, the Secretary of State must be satisfied that the purposes of the programme of work cannot be achieved without their use.

Schedule 1

Schedule 1 to the Act describes methods of humane killing. Killing a protected animal using one of the methods described in Schedule 1 is not considered to be a regulated procedure, and no project or personal licence authorities are required. However, personnel who carry out killing by a Schedule 1 method must be competent to do so, and listed in a register maintained by the Section 2C licence holder. Using a method not listed on Schedule 1, or a Schedule 1 method on an animal for which it is not deemed appropriate, constitutes a regulated procedure, and must be licensed by both project and personal licences. Sometimes, an alternative method may be specified on the relevant 2C licence, for which no additional licence authority is required. Further information on methods of humane killing can be found in Chapter 8.

Administration of the Act

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Assessment of licences and inspection of premises

The Act is administered by the Secretary of State at the Home Office, who takes advice from a number of sources as to whether and on what terms to grant a licence under the Act. The main source of advice is a network of Home Office Inspectors, who visit research establishments and study all applications for licences to work on animals to ensure the work is justified and meets the criteria laid down in the Act. Inspectors also visit establishments and conduct periodic reviews of licences to assess compliance with their terms and conditions. Any non-compliance is reported to the Secretary of State, who may issue compliance notices, revoke licences, impose retraining requirements or formally admonish the licence holder. If an inspector believes that an animal is suffering excessively they may require it to be killed immediately.

The Home Secretary may also take advice from an independent assessor, and regarding project licence applications from the Committee for the Protection of Animals Used for Scientific Purposes. This is a panel of senior researchers, legal, veterinary and medical personnel and representatives of welfare groups. The Committee provides advice on matters such as the acquisition, breeding, accommodation, care and use of protected animals to both the Secretary of State and the local Animal Welfare and Ethical Review Bodies. The committee aims to ensure that best practice is shared, and may also promote the sharing of such practices with other EU member states.

Offences and infringements

Breaches of certain sections of the Act or licence conditions may be offences under the Act, and may potentially lead to a prison sentence or a fine. Other breaches may not be offences but may be classed as infringements, and may result in variation or revocation of the licence.

The Secretary of State can issue a compliance notice, if a licence holder has failed or is failing to comply with the Act or licence conditions. He may also suspend licences if it appears to be urgently necessary for the welfare of any animal. If a Section 2C licence is suspended, animals may no longer be bred or kept there, and no further regulated procedures may be performed. Any animal that is suffering or likely to suffer as a result of regulated procedures may have to be humanely killed.

If a project licence is suspended, all regulated procedures being carried out under the authority of that licence must immediately stop.

If a personal licence is suspended, the holder may not perform regulated procedures. The responsibility for the care of the animals that have been used by that licensee must be transferred immediately to another personal licence holder with the necessary authorities.

Communication

Within a designated establishment many people are involved in caring for and maintaining the welfare of protected animals. The Act describes several persons with particular responsibilities towards animals, including the 2C licence holder, project licence holder, personal licence holder, veterinary surgeon, training officer and animal care and welfare officer. Open communication between these people is vital if everyone is to carry out their responsibilities effectively and if animal welfare is to be maintained. The animals cannot speak for themselves: they rely on personnel to attend to their needs.

Statistics of Animal Usage

The statistics from the annual returns submitted by project licence holders show that on 31 December 2011 there were 181 scientific procedure establishments in the UK, with 2624 project licences and 15 403 personal licensees covering the work that was done[7]. In 2011 3.71 million animals, of which 71% were mice and 15% were fish, were used in 3.79 million procedures. Birds were used in 4% of procedure, the majority of these being domestic chickens. Dogs, cats and primates together accounted for fewer than 7300 animals, less than 0.25% of the animals used. Genetically altered animals accounted for 54% of procedures.

Fundamental or applied studies into medical, veterinary or biological research, development and production accounted for 89% of procedures, and the remaining animals were used in toxicology.

These figures should be considered in the light of other forms of animal usage. For example, approximately 900 million poultry and 40 million cattle, calves, pigs and sheep are slaughtered for food each year in the UK[8]. The number of animals in research is

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therefore small in comparison, but nevertheless it raises ethical issues and must be conducted to the highest possible standards.

Other Relevant Legislation

There are many other pieces of legislation, regulations and guidelines which may be relevant to certain areas of laboratory animal use. The ASPA allows personal licence holders to conduct activities that would otherwise be prohibited under other legislation, protecting licence holders from prosecution. However, this protection is only effective if operating in compliance with the three licences of the ASPA. In addition, compliance with some of these pieces of other legislation (e.g. Wildlife and Countryside Act 1981) is still required for some types of work. Licence holders are required to ensure that they are in compliance with all relevant legislation. Many of these are summarised in Table 3.2. The list is not exhaustive.

Table 3.2 Legislation relevant to animal use in the UK.

Protection of animals
Animal Welfare Act 2006
This places a duty of care on an owner not to be cruel to an animal and to ensure its welfare needs are met, and includes acts of omission. It applies to 'animals that are commonly domesticated in the UK, or that are under the control of man', which includes laboratory animals. This Act applies to stock and experimental animals held at establishments.
Wildlife and Countryside Act 1981
This Act provides protection for wild animals and plants, and makes it an offence to kill, injure, trade in or take a listed species from the wild, to possess a scheduled species or to damage, destroy or obstruct access to their habitat. Working with certain wild animals requires licensing under this Act as well as under the ASPA. Licences under this Act are issued by different bodies in different countries of the UK, for example English Nature, Scottish Natural Heritage or the Countryside Council for Wales.
Veterinary Surgeons Act 1966
This requires that only a registered veterinary surgeon may carry out acts of veterinary surgery. This includes diagnosis and treatment of disease in animals.
The Animal Health Act 1981
This Act is concerned with animal disease control, such as rabies, bovine tuberculosis, bovine spongiform encephalopathy, bluetongue, swine fever, and foot and mouth. This places controls on imports and movements of various species. Many Orders under this Act impact on the use of animals in research, for example as follows. • Transport of animals is covered by the Welfare of Animals (Transport) Order 2007. This requires that animals must not be transported in a way that is likely to cause injury or undue suffering to them. • Import of live animals and products of animal origin is covered by several orders. UK import health requirements for live animals and products of animal origin are determined by DEFRA. Rabies quarantine is required for some animal species (e.g. primates). • Regulations for export of live animals and tissues may require that a veterinary check is performed to certify health of animals and fitness to travel. Exact import requirements vary widely between countries. • The Pigs (Records, Identification and Movement) Order 2011. This and other orders place restrictions on the movement of farm animal species, in order to limit the spread of animal diseases. Licensees are advised to check exact requirements for animal movement, import or export with DEFRA. Note: Home Office permission may be required to export any animal on a regulated procedure (this includes all genetically altered animals), and for acquisition of Schedule 2 animals from abroad.
Convention on International Trade in Endangered Species (CITES)
CITES is an international agreement between governments. Its aim is to ensure that international trade in specimens of wild animals and plants does not threaten their survival.
Protection of humans
Health and Safety at Work Act 1974
This Act requires that employers ensure the health and safety of workers, visitors and contractors in the workplace. Relevant regulations include the following. • Control of Substances Hazardous to Health (COSHH) Regulations 2002. These require risk assessments to be done and protective measures put in place. 'Substances' include animal allergens and pathogens. • Genetically Modified Organisms (Contained Use) Regulations 2005. These require that all activities involving GMOs must be assessed for risk to humans and the environment. The HSE must be notified of all premises using GMOs before first use. Activities are classified according to risk, Class 1 (low) to Class 4 (high), and HSE must be notified of individual activities of Class 2 to Class 4. • Ionising Radiation Regulations 1999. This includes X-rays, computed tomography (CT) scans, dual x-ray absorptiometry (DXA) scans, radiotherapy, radionucleotide imaging and administration of a radioactive substance. Employers are legally required to ensure compliance with health and safety regulations on radiation. Note: magnetic resonance imaging and ultrasound do not involve ionising radiation. • Reporting of Injuries Diseases and Dangerous Occurrences (RIDDOR). These require that certain workplace injuries and conditions, including laboratory animal allergies, are reported to the HSE.
The Veterinary Medicines Regulations 2011
These control the manufacture, distribution and marketing of veterinary medicines. This defines prescription-only medicines (or POM-Vs), which may only be given to animals under the direction of a veterinary surgeon. These regulations are updated every year.

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The Misuse of Drugs Act 1971 and Misuse of Drugs Regulations 2001
This controls the use of 'dangerous or otherwise harmful' drugs (controlled drugs), such as those which may be addictive or drugs of abuse. Drugs controlled under this Act may be classified into one of five schedules. Schedule 1 includes many drugs of abuse which are generally not used as medicines. Schedule 2 includes potent drugs such as morphine, pethidine and fentanyl. These drugs have strict requirements for storage and record keeping. Drugs in Schedules 3–5 have less strict storage and recording requirements.
DEFRA, Department for Environment, Food and Rural Affairs; GMO, genetically modified organism; HSE, Health and Safety Executive.

Legislation falls generally into two categories: laws for the protection of animals (usually administered by the Department for Environment, Food and Rural Affairs), and laws for the protection of humans (administered by the Department of Health or the Health and Safety Executive).

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Chapter 4

Balancing Animal Welfare with Science

Welfare versus Science?

Using an animal for science is strictly regulated under many jurisdictions, to protect the animal's welfare. Public opinion, in general, regards the use of animals to obtain medical benefits as acceptable provided that the animal does not suffer unnecessarily[1]. However, there exists a tension between the impact on the animals' welfare and any necessity to use them to generate scientific data.

What is Welfare?

The modern concept of welfare is the presence of positives with considerations of both positive feelings (what an animal 'likes') and resources that an animal is motivated to obtain (what an animal 'wants')[2], rather than the absence of negatives which came from the definition of good animal welfare according to the Five Freedoms developed by the Farm Animal Welfare Council[3]. These five freedoms are:

- freedom from hunger and thirst,
- freedom from discomfort,
- freedom from pain, injury and disease,
- freedom to express normal behaviours,
- freedom from fear and distress.

The modern concept of welfare that is now promoted by the Farm Animal Welfare Council[4] is that of a life worth living, from the animal's point of view, or, even better, a good life. Good standards should also apply to animals used in the laboratory, and in order to ensure that animals can be valid subjects for research it is necessary to protect their health and welfare. A judgement of the quality of life of experimental animals against the benefits to other animals, or humans, is the basis of the harm/benefit analysis. It is much more than simply the prevention of cruelty: quality of life should be used for decision-making and to do this a quality-of-life balance sheet can be prepared[5].

In the UK the Animal Welfare Act 2006 places a duty of care on owners to provide for animals' basic needs. These needs include a suitable environment, a suitable diet, to exhibit normal behaviours, to be housed with (or apart from, as applicable) other animals and to be protected from pain, injury, suffering and disease. Under this Act there are a number of codes of practice giving practical advice on how to ensure that these welfare needs are met. Current evidence suggests that welfare can be best assessed by evaluation of positive outcomes such as behavioural responses, influences on cognitive processes and physiological markers; and the explicit inclusion of positive welfare outcomes allows for analyses that are both deeper and more in tune with common sense, which can hopefully yield more objective policies following retrospective reviews.

Quality of life

An animal's quality of life has been defined as being made up of three components: its value to itself, its value to science and its value to its peer group[6]. Alternative views are that the animal has no value at all, has a value for that individual only or that its value exceeds what is 'in it' for the animal in question[7]. Any domestic species, whether farmstock, companion animal or laboratory animal, is produced by humans and so is considered by some to therefore be for human use since it would not exist if we had not arranged for it to do so7. Animals, both domestic and wild, have varied roles in society and their value can be economic, cultural, political, emotional or religious[8]. They are also attributed an inherent 'animal value' that exists by virtue of them being sentient beings. While sentience is important, it is the demonstration of rational consciousness in animals that leads to

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the ethical and welfare implications[9]. Kirkwood and Hubrecht[10] expand on the meaning of consciousness and cognition and conclude that, despite the diversity of opinion, it is of great relevance to animal welfare.

Research using animals has the ability to impact on the five freedoms, and thus on welfare and quality of life, in some of the following ways – and to varying degrees – depending on the types of procedure and the species being used:

- use of food-/fluid-regulation paradigms,
- keeping animals in metal cages on grid floors or with little bedding material,
- carrying out surgery, creating genetically altered animals that model human disease with clinical signs,
- use of single housing and restricted availability of space,
- use of husbandry methods and procedural techniques, for example ones which use excessive restraint or aversive methods (restraint can be a serious contingent harm as detailed by Hurst and West[11], who showed the level of stress caused by picking mice up by their tails).

An overview of stress

Stress is a physiological and behavioural adjustment which aims to maintain homeostasis and is part and parcel of normal life. It is possible to reduce and minimise stress levels but it has both benefits and costs to the individual. However, if stress is taken to extremes the questions are always how far, and where exactly is the cut-off point? Stress develops into distress when other biological processes, such as growth and reproduction, are compromised. When the animal is conscious of the effects on its physiological processes then it can be said to be suffering.

Stress and distress in laboratory animals may be induced by painful or non-painful stimuli, including experimental methods, environmental factors or physiological disturbances. These factors contribute to the severity of the scientific procedure and impact adversely on animal welfare. Laboratory animals have the potential to suffer in two ways and their suffering can be divided into *direct* suffering, which occurs as a result of the specific procedure carried out, and *contingent* suffering, which the animal undergoes as a result of being held in a laboratory environment[12]. For example, the way in which a test substance is administered, how much blood is taken, how the animal is restrained or trained, what anaesthesia is used, levels of post-operative care and side effects such as nausea all constitute direct suffering. Contingent suffering results from such things as housing, husbandry, transport or social hierarchy, and may include fear, boredom or discomfort, for example through living in a small cage or an abnormal social environment which impacts on the ability to exhibit a normal set of behaviours. The total effect on the animals must be assessed and take into account both direct and contingent harms.

Stress will also impact on the results of scientific procedures being carried out and therefore compromise the validity of the model being used. For example, it will damage the central nervous system at the hippocampus and basal ganglia, and it will have effects on the immune system and also on the reproductive system through effects on behavioural and social equilibria. Stress is a measurable phenomenon and can be quantified by examining various parameters of welfare. However, some parameters are multifunctional (e.g. cortisol) and some exist in chronic states and may confuse the picture (e.g. hypocorticolism).

Why assess welfare?

There are several reasons to assess welfare of animals: to ensure that the three Rs are being effectively implemented and to be able to justify the harm/benefit balance at ongoing and at retrospective reviews. It will also ensure scientific model validity, since an animal with poor welfare will not be a valid model due to the changes from normal physiology that can affect the results being measured. Records of welfare assessment can be used to reassure the general public that work is being carried out to the highest standard, frequently funded by the public purse. This is useful in ensuring good public relations and is in line with the concept of transparency and openness in research. It can also be done purely out of academic interest and welfare science is being taken increasingly seriously by the scientific community.

Welfare may be assessed qualitatively or quantitatively, but the use of objective measurements of suffering will facilitate evaluation of responses to treatment and enable decisions about changes in management strategy or the timing of euthanasia to prevent further suffering. They can be used for monitoring single incidents but should also be used to reflect the cumulative suffering and the lifetime experience of the animal. This will include direct suffering from any procedures as well as contingent suffering as a result of, for example, transport, housing or the environment. Attending to the welfare needs of animals is not a passive process; it requires a continuous cycle of planning, implementation, assessment and revision. The assessment of welfare facilitates not only the retrospective illustration of cumulative suffering and the impact of enrichment, refinement and procedures, but also helps enable the projection of future harms to the animal of both contingent and direct suffering related to anticipated change, or lack of change. The level of harm, including both contingent and direct suffering, must be weighed against an honest and

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realistic assessment of potential benefits of the programme of work.

At the heart of the legislation controlling the use of animals in science is the harm/benefit balance (see Chapters 2 and 3) which is considered at ethical review to inform the decision whether or not the work is justified. The level of harm reflects the welfare of the animals used in the experiments and by applying the R of refinement it is possible to implement many small changes to procedures that can summate to a big improvement in the animal's welfare, and thus improve the justification for its use. All research establishments are required to employ a veterinary surgeon to provide care and advice on the health and welfare of the animals being used, but this can create a potential dilemma, and put the vet into a difficult position, since in the laboratory the experimental animals' welfare inevitably has the potential to be compromised. The vet must be confident that the quality of the science has been adequately balanced against the compromise to welfare, and there are occasions when that is challenging if refinements are not being implemented vigorously[13]. This can then lead to questions about the extent to which the animals' welfare is affected and it is then necessary to demonstrate quantitative assessment of welfare rather than rely on subjective views on how the animal may be feeling. It is also beneficial to demonstrate the true welfare implications of research by monitoring animal welfare as part of the process of estimating the harms to the animals of the programme of research and being able to demonstrate the ways in which these harms can be reduced via application of the three Rs. The welfare of such animals must be maximised so that they suffer as little as possible during any experimental use. It is thus a requirement to assess their welfare.

Who should assess welfare?

Advice on health and welfare and its assessment should primarily be the responsibility on the veterinary surgeon. However, others can, and should, be actively involved in the collection of the objective data, including the research staff, the animal care staff and those with specific experience with the species, such as a zoologist or a primatologist. The evaluation of pain and distress is complex because thresholds and manifestations of pain and distress can vary between species and between individuals within a species. The first stage in assessing an animal's well-being is to become familiar with its normal appearance and behaviour. When doing 'hands-on' training in the animal house it is important to allow enough time to observe the animals that will be used in order to become familiar with their normal behavioural repertoire. Research staff should find out what the normal behaviour range is for the species they will be working with, remembering that this may not be the same as the behaviour observed in the laboratory due to the constraints of the captive environment (see www.ratlife.org). Animals have not evolved to live in cages.

How to assess welfare

Subjective evaluation of welfare

Welfare can be assessed on an *ad hoc* basis using clinical judgement and experience, but this is open to challenge by others who may lack the relevant experience and intuition. An animal's response to reduced welfare will be modified by a number of factors. Interpretation of the response will need to consider the following points.

1. The *individual details* of the animal such as its species, age and origin. These factors will all affect its response to painful stimuli. For example, a rabbit is far more stoical than a dog since a prey species will be much better at hiding illness or injury than a predator species as this will be better for its survival, since predators will look for signs of weakness when hunting.

2. The *history* of the animal and the establishment. This will need to take into account previous problems encountered, the course of the current problem, the environment in which the animal is kept, the procedures that have been carried out and any known current disease problems.

3. *Clinical examination* of the animal to assess its current condition. The extent of this will depend on the species. For example, take note of feeding behaviour (quantity, pattern of feeding), physiological signs (such as heart rate, respiration rate, body temperature, muscle tone, colour of mucous membranes) and possibly biochemical signs (such as enzyme levels). If there may be intercurrent disease, veterinary advice should be sought and taken.

4. *Mental state.* The animal may appear dull, depressed, aggressive or hyper-excitable. Particularly important are how such traits are at variance with its usual behaviour. The technician in charge of the animal will often be the best person to observe any changes in its temperament or its reluctance or otherwise to accept handling.

5. The level of *activity* of the animal may range from total inactivity to maniacal hyperactivity. Particular attention should be given to noticing any changes in gait, posture or facial expression. Interpretation of behaviour may require specialist advice. For example, see Langford et al.[14] for details on interpretation of facial expression in mice.

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6. *Vocalisation* will depend on the species and there are a wide variety of different noises produced by each species. The sound emitted may be outside the human auditory range and therefore go unnoticed but may be causing distress to others of the same species.

7. *Response to analgesics.* If a dose of an analgesic drug is administered and the animal's condition and demeanour improves, then this can be used as evidence that pain was indeed present.

All these are very general descriptions on how to assess changes in welfare qualitatively, and the interpretation of such parameters will vary quite widely between observers depending on their knowledge and experience of the species and the individual animal under observation. In making the judgement it is necessary to consider the species, whether it is juvenile or adult, any normal physiological variation (e.g. pregnancy) and the animal's individual temperament.

Thus the assessment of pain and distress in animals can be problematic, since it can be subjective and may be based on anthropomorphic assumptions that things which are distressing for humans will also be distressing for animals, which is not necessarily the case. This may lead to a tendency to overestimate the pain and suffering experienced by animals in some situations, and underestimate in others. Since this type of assessment is based on the subjective judgements of individuals it is likely that two people with different backgrounds and experiences observing the same animal will give different scores to the level of welfare of a particular individual animal. This can be avoided by having several observers score the animal at each point and averaging the scores, or by having all assessments performed by just one, or a few, experienced persons. Vets, researchers or animal care staff can, with experience, become consistent in their markings and reduce this variation between assessors. It is also necessary to differentiate between what is normal and what is normally *seen*, which may be quite far from normal. Sometimes quite abnormal states may be classified by some observers as normal, for example stereotypic behaviours in captive animals are frequently misjudged as normal because they occur commonly.

Implementation of humane end points

For both ethical and experimental reasons it is important to keep adverse effects to a minimum. Objective methods of assessing pain and distress in animals are valuable for several reasons: they allow potential refinements and new research techniques to be evaluated critically, they allow real judgements to be made about the need for and efficacy of analgesics and they allow the implementation of humane end points. This ensures a defined set of criteria at which point the animal is taken off study, usually by euthanasia, but sometimes simply by withdrawal from the procedures and release from further study, when the welfare has become compromised beyond a certain point. This ensures that unnecessary suffering beyond that level does not occur.

Objective evaluation of welfare

It is much better to consider how to quantify the assessment being made so that it is objective, in order to be able to judge whether welfare has been improved, to ascertain whether the degree of pain is within the severity classification of the project and to remove any inter-observer variability in making the assessment. The data collected must be robust so as to enable consistency over time and across groups and between different observers. The data may involve the use of scoring sheets and checklists. It is necessary to be aware of natural differences such as species differences, age or developmental differences and differences in sex and reproductive status and in social status.

The classical simple distress scoring (or welfare assessment) system is based on that of Morton and Griffiths[15] (see General distress scoring sheet, Figure 4.1). Using this scheme monitor:

- appearance,
- food and water intake,
- clinical signs,
- natural behaviour,
- provoked behaviour.

FIGURE 4.1 General distress scoring sheet.

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PARAMETER	ANIMAL ID	SCORE	DATE/ TIME	DATE/ TIME
APPEARANCE	NORMAL	0		
	GENERAL LACK OF GROOMING	1		
	COAT STARING, OCULAR AND NASAL DISCHARGES	2		
	PILOERECTION, HUNCHED UP	3		
FOOD AND WATER INTAKE	NORMAL	0		
	UNCERTAIN: BODY WEIGHT ↓<5%	1		
	INTAKE: BODY WEIGHT ↓10–15%	2		
	NO FOOD OR WATER INTAKE	3		
CLINICAL SIGNS	NORMAL T, CARDIAC AND RESPIRATORY RATES	0		
	SLIGHT CHANGES	1		
	T ± 1°C, C/R RATES ↕30%	2		
	T ± 2°C, C/R RATES ↕50% OR VERY ↓	3		
NATURAL BEHAVIOUR	NORMAL	0		
	MINOR CHANGES	1		
	LESS MOBILE AND ALERT, ISOLATED	2		
	VOCALISATION, SELF MUTILATION, RESTLESS OR STILL	3		
PROVOKED BEHAVIOUR	NORMAL	0		
	MINOR DEPRESSION OR EXAGGERATED RESPONSE	1		
	MODERATE CHANGE IN EXPECTED BEHAVIOUR	2		
	REACTS VIOLENTLY, OR VERY WEAK AND PRECOMATOSE	3		
SCORE	IF YOU HAVE SCORED A 3 MORE THAN ONCE, SCORE AN EXTRA POINT FOR EACH 3	2–5		
	TOTAL	0–20		

JUDGEMENT
0–4 Normal.
5–9 Monitor carefully, consider analgesics or other treatments.
10–14 Suffering, provide relief, observe regularly. Seek second opinion from animal care staff and/or designated veterinary surgeon. Consider termination.
15–20 Severe distress, is this severity level justified?

At the start of the assessment view the animal from a distance and note its natural appearance and undisturbed behaviour. Next, as you approach the animal it should take notice of you and interact in some way. The nature of that interaction can be used to assess whether the animal is responding normally. Finally a detailed clinical examination can be carried out making appropriate clinical measurements (such as body weight).

If there is no deviation from the normal, for each parameter score 0. If there is mild deviation score 1, moderate deviation score 2, and substantial deviation score 3. If three is scored more than once, give an extra 1 to each, making a maximum score of 20.

The use of such a system encourages regular close observation of the animal, leading to improved standards of animal care. If the animal is found to be deteriorating then actively consider euthanasia before the experimental end point is reached. It is vital to remember to re-score the animal after giving analgesics or other treatments to ensure that the drugs have had the desired effect and the animal's condition has improved. Specific scoring systems have been devised to examine many species and types of experiments in order to define an intervention point such as provision of analgesia or carrying out of euthanasia, such as the Mouse Grimace Scale14.

Frequency of assessments

The frequency of use of the scoring system to assess the animal will depend on when signs are expected to occur and the length of time that they will persist. For example, in a toxicity study the early signs are likely to be due to the primary effects of a substance on a particular tissue or cell, which depends on the physical and chemical characteristics of the test substance.

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Secondary effects occur after the compound has been taken into the body and undergone metabolism in the cells. The effects of this may occur at cellular level, organ level or affect the whole animal and may be manifested in a variety of physiological and behavioural effects. Tertiary effects may not be related directly to the interaction of the compound itself within the body; for example, if a compound induces diarrhoea the animal may become dehydrated if it does not drink, and it is the dehydration which will cause the death of the animal. Signs that occur shortly before death may be due to the secondary effects of the substance but are more likely to be due the tertiary effects. Carcinogenicity studies may involve a necessary assessment of tertiary effects and provide needed experimental data, but the majority of toxicity studies are to determine the primary and secondary effects; the tertiary effects provide little additional information. The combination of the type of clinical signs and their duration can be used to assess the severity of the procedure and the intensity of suffering of the animal. The observations should therefore be frequent enough to identify any animals that are showing the defined set of signs of distress to enable euthanasia to be carried out in a timely manner. It is also scientifically useful to collect as much data as possible on clinical signs and any unobserved animal deaths will represent lost information on the compound under test and a reduction in the quality of the scientific information yielded.

Development of welfare assessment systems

In practice, scoring systems can be valuable tools in keeping the harm to animals in biomedical research to a minimum. However, the system outlined in Figure 4.1 is very general. Many research projects produce changes in just one organ system, and a general scoring system such as the one described here may not be sensitive enough to pick up specific indicators of poor welfare in these cases. To overcome this the parameters are modified to include only those specific changes which are anticipated in the particular project. Pilot studies are important to develop these more specific assessment sheets and it may be necessary to modify the chosen system as the experiment progresses. The list of clinical signs to be assessed for a particular scientific procedure should be developed through experience and to a large extent will be unique to the specific husbandry system, the species or strain and to the specific experiment.

A scheme of scoring clinical signs for recognition and assessment of adverse effects on animals during scientific procedures has been shown to have a number of benefits, including the following.

- Closer observation of animals by all staff at critical times in the experiment. This increases awareness of the potential adverse effects and allows for the appropriate use of methods of alleviation.
- Subjective assessments are avoided and opinion is both more structured and more evidence-based, thus promoting more fruitful dialogue between scientists and animal care staff.
- Providing assistance with making a valid harm/benefit analysis in the justification of a procedure.
- Consistency in scoring is increased.
- Combinations of signs can be used to indicate the overall severity of the procedure and the continued assessment will determine the effectiveness of any therapies given to alleviate the adverse effects.
- The assessment will allow retrospective review of procedures to assess which experimental models cause the least pain and distress. This will therefore help to further refine procedures in the future, to develop better systems of animal care, yielding better science and better animal welfare.
- The use of scoring systems assists with the training of new personnel in how to assess adverse effects on animals.

There are various components to the objective assessment of an animal's welfare, as follows.

Behavioural: quantified behavioural measurement with systematic sampling allows monitoring of change. Data are collected on the duration of behavioural states and grouped into functional categories for analysis. The incidence of abnormal behaviours, such as stereotypic behaviour, inappropriate time budgeting such as overgrooming, the presence of neophobia and/or depression, are all parameters to be measured. Behaviour analysis is complex and involves assessment of a wide range of behaviours depending on the species and the situation. Figure 4.2 is an example of a behavioural ethogram, listing some of the parameters that can be assessed in primates. Normal does not necessarily mean natural and it is necessary to be familiar with what is natural for the particular species. This must include sufficient time to observe animals and become familiar with their behaviour patterns. Only with that background is it possible to recognise the onset of stereotypic behaviour which is a form of behaviour induced by chronic frustration[16], possibly in response to a barren environment. It may manifest itself as extremes of behaviour (such as pacing, or repeated circling, head turning or self-harming) or as early signs of behavioural disturbance with a significant shift in the range and frequency of behaviours and the development of unusual, non-functional behaviour. The goal is to ensure that animals have a natural frequency and range of behaviours and to take action to expand the behavioural repertoire by providing adequate environmental enrichment. Behavioural observations can be made with different recording methods including focal animal sampling, scan

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sampling, all occurrences of a specified behaviour and/or ad-lib sampling[17]. The data can be used to quantify welfare and can be reviewed and reassessed at regular time points. The collection and review of such data can encourage a team approach to action on the animal's welfare involving scientists, the vet, the animal technicians and specialist animal behaviour personnel working together. Results can be used in communications used in public education and to support applications for funding.

Physiological: this component covers autonomic parameters such as heart rate, respiration rate, blood pressure and/or temperature.

Hormonal measurement: this covers hormones such as hypothalamic–pituitary–adrenal axis (HPA) hormones, including the stress hormone cortisol, in blood, faeces, urine, saliva, tears or hair. One difficulty in the measurement of cortisol can be the interpretation of results.

Immunological: the neutrophil activation assay[18] or measurement of leukocyte cell counts can give an indication of the effect on the immune response. The measurement of acute phase proteins can also be applied to the assessment of welfare[19].

Physical condition:

Body condition: this includes body weight, which must take into account age, sex and reproductive condition, development/reproductive condition and condition scoring (see Figure 4.3)

Alopecia scoring: trichotillomania in humans is an example of obsessive compulsive disorder, associated with psychological stress and relieved by anti-depressants. It is affected by hormones (e.g. prolactin and melatonin), disease and nutrition, ageing and reproductive condition. In primates, for example, it is a pathological intensification of natural grooming behaviour, frequently with hair ingestion, and is a symptom of psychogenic maladjustment to a poor environment, which can be reduced by the use of various types of environmental enrichment. It can be scored to assist with welfare assessment as shown by Honess et al.[20].

FIGURE 4.2 Example of a behavioural ethogram listing some of the parameters that can be assessed in primates.

a	Avoid aggression		
b	Being groomed	m	Manipulate cage
c	Presenting	o	Observing
d	Drink	p	Play
e	Eat/forage	q	Stereotypic behaviour
f	Aggression	r	Rapid energy expediture
g	Urinate	s	Self groom
h	Hug	t	Play with object
i	Inactive/resting	v	Defaecate
j	Playfight	w	Infant care
k	Self harm	x	Return aggression
l	Locomotion	z	Hidden from view

FIGURE 4.3 Body condition scoring for mice.

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	**Body condition score 1** Animal is emaciated Skeletal structure extremely prominent Little or no flesh cover, vertebrae distinctly segmented
	**Body condition score 2** Animal is under-conditioned Segmentation of vertebral column evident Dorsal pelvic bones readily palpable
	**Body condition score 3** Animal is well conditioned Vertebrae and dorsal pelvis not prominent Dorsal pelvic bones palpable with slight pressure
	**Body condition score 4** Animal is over-conditioned Spine is a continuous column Vertebrae only palpable with firm pressure
	**Body condition score 5** Animal is obese Appears smooth and bulky Bone structure disappears under flesh and subcutaneous fat

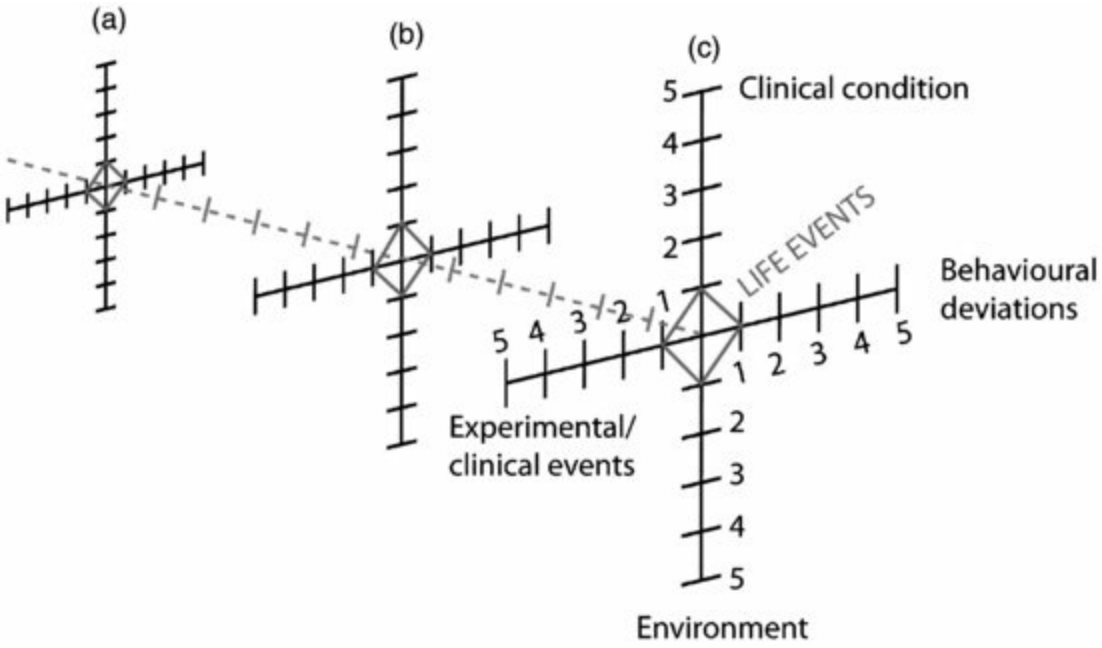
Consideration of cumulative suffering

EU Directive 2010/63 refers to '… taking into account the life-time experience of the individual animal …' (para. 25), '… to enhanc[ing] the life-time experience of the animals …' (para. 31) and '… reduc[ing] the duration and intensity of suffering to the minimum possible …' (article 13.3b). Experiments are assigned a severity category which shall take into account the nature of pain, suffering, distress and lasting harm, its intensity, the duration, frequency and multiplicity of techniques and cumulative suffering within a procedure (Annex VIII).

Combining a range of assessment parameters into one usable entity has been identified as an important goal in providing a practical, objective and robust assessment of welfare. The Extended Welfare Assessment Grid[21], Figure 4.4, provides for the incorporation of changes in the state of an animal over time allowing for predictive, retrospective, or scheduled, event monitoring. It enables the numerical, as well as visual, representation of the animal's welfare so this can be considered in the context of the careful and realistic justification for experimental use of the animal. This assessment method represents a valuable tool for those tasked with ensuring ethical oversight as well as for those planning the use of, or monitoring, animals in research. It is particularly applicable to animals used in long-term studies. This system draws attention to the temporal component of suffering that is often overlooked in the planning of research schedules and allows an assessment of cumulative suffering imposed by the events that occur.

FIGURE 4.4 Extended Welfare Assessment Grid, from Honess and Wolfensohn21.

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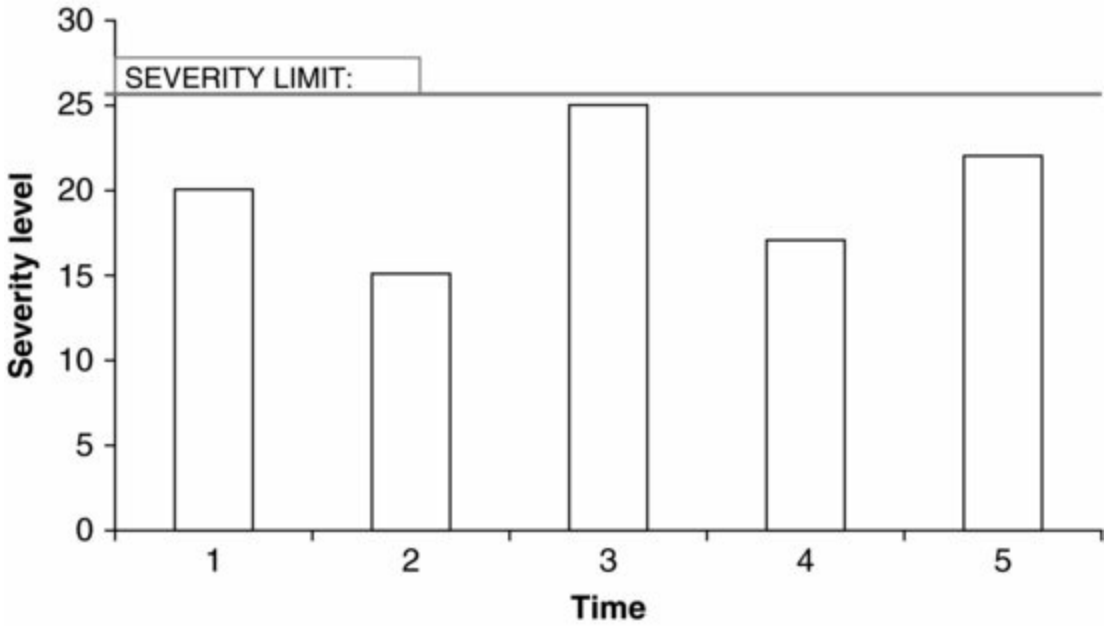


EU Directive 2010/63 requires that procedures are classified according to severity level as being of mild, moderate or severe. However, these are subjective terms open to interpretation. Without a supporting structure and scales of reference, these terms are of limited operational value since some researchers continue to use purely subjective assessments which are open to debate, or no method of assessment at all. Pain and distress caused by scientific procedures is predictable and should be easily avoided or relieved, but even if there is unexpected distress, objectively measuring pain and distress in animals is not impossible. The challenge is for us to decipher the state of the animal from the information available. If any doubt exists then the welfare of the animal must come first, and the responsibility for this lies with the researcher.

An animal may suffer as a result of a procedure that is carried out at a certain point in time but then it may undergo another procedure or repeated procedures over and over again (see Figure 4.5). Although each individual procedure may not go over the severity limit, the actual level of suffering may not be higher for each individual episode as the animal may be become habituated to the procedures (such as handling or restraint) or it may become hyper-sensitised as it learns to anticipate what is about to occur and becomes fearful, thus increasing the amount of suffering with each event. It is, however, impossible to simply add up the subsequent events to assess cumulative suffering. The important thing from the animal's point of view is, it is getting better, or is it getting worse? How long is it going to go on? Also, from the ethical perspective, is the level of cumulative suffering justified by the benefits accrued from the scientific data collected?

FIGURE 4.5 Repeated procedures carried out on the same animal.

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It is important to remember that the assessment of welfare alone is not sufficient. Proper welfare assessment allows feedback on changes affecting the animal's welfare, but if this is simply an academic exercise it does nothing for the animal. Decisions must be taken. The outcome could be to do nothing but, if so, there must be reasons why. Although we wish to evaluate the animal's quality of life, the animal's perception of its own welfare is not affected by the reason it is maintained (whether it is for experimental or breeding use, for example) or the cause of its suffering[22]. Welfare assessment can be used to demonstrate the true implications of research and the effects of the implementation of refinements but the outcome of monitoring and assessment must be action to improve welfare; welfare assessment is simply the tool to demonstrate the action is effective. All those who interact with laboratory animals must accept responsibility in delivering animal welfare; monitoring is simply the tool that demonstrates whether it is working or not.

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Chapter 5

Animal Husbandry, Environmental Enrichment and Occupational Health and Safety

Animal Husbandry

Experimental animals are a part of the experimental system, so it is important to minimise variation between animals in a study (see Chapter 6). Animals that give reproducible results in experiments should therefore be raised under standard conditions which keep the animals free of unwanted disease and stress and maintain their defined microbiological status. Any recommendation affecting the health or husbandry of the animals must therefore be considered together with the science that is being carried out. Housing animals in a good-quality environment will result in better experimental models in a range of different areas of research and testing. In toxicology, the old belief that the best way to standardise results is to house animals in barren conditions has been repeatedly challenged in the scientific literature, which shows that stress and anxiety due to inappropriate husbandry will affect animals, and therefore data, in unpredictable ways. Just as most scientists will accept and use models that have been improved by using new equipment, techniques, reagents or strains, they should also accept and use models that have been improved by refining husbandry: the principle is exactly the same[1].

Laboratory animals do not have the freedom to move away from adverse conditions, to search for food and water or to find a better nesting site. All their physiological and behavioural needs must be supplied by the laboratory environment. These needs are not optional: they must be catered for or poor health will result. Following the application of the five freedoms[2] – which are reiterated in the UK Animal Welfare Act 2006, which protects all laboratory animals, in addition to the Animals (Scientific Procedures) Act 1986 – the freedom from hunger and thirst means there must be optimum diet supplied to ensure growth, reproduction and longevity and free access to food and water. Freedom from discomfort means there must be adequate shelter and warmth, and a comfortable resting area. Freedom from pain, injury and disease means that disease and injury must be prevented and treated where necessary. Freedom from fear and distress means the conditions must avoid mental suffering; and freedom to express normal behaviour means there must be adequate space to allow a normal range of movement, without overcrowding, and social company.

Guidelines on the care of laboratory animals are published in Appendix A to the 'Convention on the protection of vertebrate animals in experimental and other scientific procedures'[3]. For those in the USA or in institutes working to the Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC) standards, the *National Research Council Guide for the Care and Use of Laboratory Animals* is the accepted text[4]. These documents give details of recommended environmental conditions, and include minimum cage sizes and stocking densities.

In addition to considering food, bedding and water provision, and the type of caging in which the animal will be kept, the training and experience of staff, handling methods and the overall environment, such as the level of background noise, can all have an effect on the animal, which may be manifested in some way that affects the study being carried out.

Types of housing

The microbiological status of an animal will depend on the level of control that is exercised over its exposure to different organisms. Housing for laboratory rodents, rabbits and some larger species is now usually designed in such a way as to offer a barrier for microbiological containment. This may be done because it is required as a strategy for the containment of animals which may pose a risk to human safety, because they are carrying biological agents that fall into hazard groups categorised by the Advisory Committee on Dangerous Pathogens. Sometimes the principal requirement is to protect staff from animal allergens, or it may be to prevent the microbiological spread of organisms between animals in order to maintain their health status, perhaps to allow animals of different microbiological backgrounds to be housed in close proximity. There are various terms used to describe

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the microbiological status of laboratory animals (e.g. conventional, or specific-pathogen-free (SPF); see the section on Sources of infection in Chapter 6), which in turn indicates the type of housing in which they are kept in order to ensure adequate biosecurity to maintain that level of microbiological status. The biosecurity barrier can be at the level of the cage/pen type and also at the level of the type of facility. Cages must be made from harmless material which is easily cleaned and sterilised, and must be able to withstand attempts to escape. The following descriptions are in increasing order of biosecurity.

Conventional

In conventional housing animals are housed in cages or enclosures which are open to the atmosphere of the room or building. For rodents, polycarbonate shoebox cages with a wire lid on open shelving or racks are relatively inexpensive, durable and easy to clean, but provide no barrier to microorganisms and therefore animals are at risk of exposure to infectious agents. Larger animals are often held in conventional facilities.

Filter-top cages

Cages are held on an open rack, but covered with individual filters above a conventional wire lid. Some containment of microorganisms is achieved with well-fitting lids, but the microenvironment in the cage is compromised as there is restricted airflow. The barrier remains at cage level and relies on an air-tight seal and passive movement of air through the filter. The filter top should only be removed in a cage-changing station/safety cabinet, which can make them difficult to work with.

Ventilated cabinets

These are similar to sealed cupboards with their own filtered air supply. The cabinet operates under negative pressure. Inside, filter-top cages are generally used. They are easy to install, relatively cheap and mobile but can present a risk when the cabinet doors are open or at cage changes.

Individually ventilated caging systems

Increasing use is being made of individually ventilated cages (IVCs) for laboratory rodents, to keep the animals at a defined health status. These cages are usually of the shoebox type, made of polycarbonate plastic, but they have filter tops held in a specialised rack that allows each cage to receive its air supply individually. Air is forced into or drawn out of the cage through nozzles adjacent to the lid. Cages can be held at positive or negative pressure, depending on the nature of the barrier to either reduce risk of pathogen contamination or to provide containment. The pressure is variable and is determined by whether or not the cage is sealed. Air movement through the nozzles produces sound, but in general the lower the air speed the lower the noise level is likely to be. The background noise and vibration in such units has been found to have an effect on some strains of mice[5]. The fans and filters used may be connected directly to the cage rack within the animal room, or may be sited remotely within a service plant room. If fans are situated on the cage rack, vibration may be transmitted to the cage. Whatever the design, it is crucial to install an alarm system, and to consider procedures in the event of a breakdown or power failure.

The containment provided by the cage system must be maintained while the cage is cleaned or the animals examined. These procedures are therefore carried out in ventilated workstations or safety cabinets. There are three classes: class I protects the operator, class II protects the animal and the operator and has an easy-access open front and class III is, in effect, a totally sealed box. IVCs are complex cage systems that rely on engineering to support the animals' environment. If this equipment is to fulfil its objectives thorough staff training is required, ranging from simple husbandry procedures to prevent damage to cages, to knowing how to ventilate a rack of cages when a fan unit breaks down since such failures can lead to risk of suffocation. Standard operating procedures are required for IVCs and safety cabinets to ensure that the required level of containment is maintained.

The use of IVCs is increasing as they provide protection of valuable transgenic lines of mice from environmental pathogens, and can increase room capacity. The cages also require less frequent changing. The system allows for containment of animals that are in quarantine or are infected, and are easier to work with than isolators. They also provide protection of staff from allergens. The IVC provides the animal with an environment that is controlled for ventilation, temperature and relative humidity. The containment provided by an IVC may be beneficial: the quality of the science may be improved by using healthier animals, enabling fewer animals to be used more effectively. There are drawbacks, however, since this type of caging makes the animal more remote from the staff. The animal technician cannot see the animal as easily, and cannot hear or smell the animal, all valuable sources of information when assessing its overall condition. The welfare of the animals will depend on the qualities and skills of the staff employed. Their success relies on ‘operating procedures’: and there is a risk of cross contamination, for example, at cage

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servicing[6].

Flexible film isolators

These are used mainly for small rodents but sometimes also for gnotobiotic or germ-free large animals (pigs, poultry, calves). An isolator is like a large plastic bubble, which is sealed from the outside world. Filtered air is supplied, and equipment and food are passed in through a port which allows disinfection of the packaging prior to entry into the isolator. Isolators operate under negative or positive pressure, offering complete environmental protection against the spread of pathogens into or out of the isolator, so animals of different health status can be housed in the same room, in different isolators. They can be used for containment of animals with zoonotic pathogens; for example, Advisory Committee on Dangerous Pathogens category 3, protection of valuable or immunodeficient animals and for quarantine of animals from various sources. They are mobile but do not make good use of available floor space. They must be alarmed in case of failure of the air supply and they can be difficult to work with.

Bedding and nesting materials

Bedding material needs to fulfill a number of requirements. It must provide warmth and be comfortable for resting, and must absorb urine. It should be non-toxic, not dehydrating for neonates and relatively dust-free.

Many animal species need to be in control of their microenvironment. They huddle and build nests in order to adjust the temperature, humidity and light, all of which will impact on the animal's physiological and psychological status. Cages or pens should therefore have a solid floor with bedding for digging, nesting and resting, and the animal should be able to create separate spaces for sleeping, defecating, urinating, eating and playing. What is important about a cage or pen is not its size but its complexity. Sawdust is a popular choice for bedding material, but fine particles can cause preputial and respiratory problems. Nest boxes may be provided, but animals may need additional nesting material. Paper towels, tissues and wood wool allow the animal to regulate microclimates, create a shelter to hide and control the social environment. Use of used nesting material, rather than bedding material, can reduce aggression. Softwoods such as pine should be avoided as they contain hydrocarbons that may induce hepatic microsomal enzymes[7].

Nutrition

Diet

Any laboratory animal must be fed an adequate diet that is nutritionally complete in an appropriate quantity. Overfeeding will lead to obesity which is associated with an increased incidence of tumour development, whereas calorie restriction has been shown to increase longevity. Even when on maintenance diets, rodents show a tendency towards obesity[8]. High-protein diets have been associated with renal failure. Consistent composition and quality of the diet are essential to reduce experimental variability. The food must be palatable, which will depend not only on flavour (low-fat food is less palatable) but also on its physical form, such as the hardness and size of the pellets. The composition of diet can affect the experimental outcome. In toxicity testing the diet may bind toxins, and may alter gut microflora and drug metabolism. The toxins given to the animals can also affect the diet by degrading constituents, suppressing appetite and/or reducing palatability. Other points to note about diet are that the heat treatment used in production of extruded or expanded pellets will destroy most bacteria. If further sterilisation is required it can be done by irradiation or autoclaving. Diets have a limited shelf life and must be stored correctly to ensure they do not deteriorate.

Water supply

Bacterial contamination can be controlled by acidification or chlorination. Trace elements, heavy metals and organic chemicals can be removed by reverse osmosis, de-ionisation or microfiltration. Automatic watering systems may appear to save time and ensure a constant water supply, but in solid-floored systems care must be taken to prevent flooding. Water pressure must be monitored continuously to ensure that the flow is adequate. It is necessary to avoid both flooding and dehydration and to clean the central reservoir regularly and check for contamination with bacteria and fungi. All watering systems need to be checked and cleaned frequently.

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Environment

The Home Office Codes of Practice[9,10], which will be updated during 2013–2014, and EU Directive 2010/63, Annex III, set out minimum standards. The macroenvironment is the set of conditions outside the cage which can impact on the animals, and the microenvironment is the set of conditions within the cage, which may be very different to those outside. It is important not to ignore the microenvironment which is critical for the animal, sometimes there is a tendency to just concentrate on and monitor the macroenvironment.

Temperature and relative humidity

The most important of environmental factors is temperature. Animals will respond to changes in temperature by altering their metabolic rate so changes in ambient temperature can affect experimental results by altering drug activity, toxicity and metabolism; feed and water intake; susceptibility to disease; and reproductive performance and growth. All of these may affect experimental data being collected. Factors which affect the temperature in the microenvironment include the cage type (metal/plastic), whether or not it has a filter top, the presence or absence of bedding or nesting material, the housing density in the room (as more animals generate more heat) and the presence in the room of other equipment that may generate heat. Temperature regulation is also affected by relative humidity which can be difficult to control, especially in winter. The specified level is 55±10%. Humidity outside this range will predispose to disease: respiratory disease in rats or nasal dermatitis in gerbils can occur if humidity is high, or ringtail may arise in rodents if it is low. Temperature and humidity should therefore be regulated to keep within the prescribed limits. Most modern animal units have a Building Management System to record temperature, provide supplementary heating or cooling and regulate ventilation, which will influence temperature, humidity and gaseous and particulate contaminants aiming to reduce the level of smells, dust, gases (e.g. ammonia), infectious agents and allergens. Inadequate ventilation leads to rises in ammonia, carbon dioxide and humidity plus dust if poor-quality bedding is used. Ventilation rates should be related to stocking density and heat generated by equipment in the animal rooms, and can be used to create differential pressures. A minimum of 15–20 air changes per hour is recommended for rodents, but achieving these rates at the room level does not guarantee adequate changes at cage level and does not necessarily ensure an adequate microenvironment.

Noise

Excessive background noise or intermittent loud noise should be avoided as it can cause poor reproductive performance, audiogenic seizures (in gerbils, DBA2 mice or Lister hooded rats) and an abnormal response in studies involving stress. Rodents can hear high-frequency ultrasound not audible to humans, such as that emitted by computers (20–30 kHz).

Light

Lighting can have a major influence on an animal's physiological and behavioural responses in two ways, as follows.

1. *Photoperiod*: the light/dark cycle regulates the circadian rhythm and reproductive cycles. A 24 h cycle of 12 h:12 h or 14 h:10 h is normal for rodents. Changes in proportion will affect gestation period, oestrus cycle and time of ovulation. Interruptions during the dark cycle are particularly disruptive of circadian rhythms and oestrus cycles and can lead to enhancement of tumour growth.

2. *Intensity*: 'normal' office light levels are 300–400 lux but light intensities above 60 lux can lead to retinal degeneration in albino animals. Cages on the top of a rack may be exposed to much higher levels of light than those below and should always be provided with a shelf over the top. Aim for an intensity of 200 lux 1 m from the floor and intensity of 25 lux at cage level by providing boxes, tubes or igloos for sleeping, tinted cages and turning down lights when not working in the room.

Animal rooms generally do not have windows since their presence will lead to variation in light intensity, photoperiod and temperature, and possible lapses in security[11].

Handling techniques

Good animal handling techniques will reduce the risk of injury from bites and scratches and will increase the confidence of both the handler and the animal, thus reducing stress to all involved. All animals will respond in some way to the presence of a human and most species can recognise individuals and will be nervous of strangers. It is therefore important for the researcher to establish a friendly relationship with the animal to reduce nervousness on both sides. An animal that is confident and relaxed with its

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handler will be more co-operative, enabling procedures to be carried out more easily. Some aspects of handling will vary according to the species.

Environmental Enrichment

Animals in the wild will find and process food, build nests, defend their territory and socialise, whereas animals in captivity in the laboratory have little opportunity to pursue such behaviours. This potentially results in adverse effects on their welfare and physiology. It is therefore necessary to make alterations to the environment which enhance welfare and increase the frequency and diversity of natural behaviours. For all species the enrichment strategy should permit socialisation, preferably direct but at least visual, the accommodation should provide an interesting, stimulating environment, there should be variation in textures and taste in feeding and foraging, and the animal should have access to some manipulative objects. In order to be classed as an 'enrichment' it must increase the frequency and diversity of positive natural behaviour, decrease frequency of abnormal behaviour, maximise utilisation of the environment and increase an animal's ability to cope with the challenges of captivity.

However, the provision of environmental enrichment can cause controversy, because even though an enriched environment may produce more 'normal' animals and enhance their welfare, it may also change their biological characteristics and may increase variability. It must therefore be compatible with the study. In general, captive domesticated rodents need:

- properly structured space,
- solid floors with litter,
- group housing for social animals,
- refuges,
- nesting material,
- treats for foraging,
- positive (or less negative) interactions with humans,
- an appropriate duration and intensity of light and dark.

The provision of environmental enrichment for animals held in the laboratory is now accepted as standard practice. However, it is important to ensure that any enrichment provided is actually beneficial to the animals, rather than just a token gesture. One must therefore evaluate the enrichment and know what the animals wants and what it needs, and to satisfy those desires as much as possible. Many people have spent a great deal of time and effort on developing environmental enrichment for laboratory animals and refining other aspects of animal housing and care, only to find that their improvements are not implemented because they are not considered 'scientifically validated' or are unacceptable for other reasons. This problem can be overcome by a structured, scientifically robust approach to evaluating enrichment and other husbandry refinements. Providing a good-quality 'home' environment is an essential component of ensuring good laboratory animal welfare. A suitable environment will allow the animals to perform a range of natural behaviours including social behaviours, and different sorts of locomotion, exploration, hiding and foraging. It can also distract them from any pain, discomfort or distress they may experience and improve the ability to monitor them since, if animals can display a broad range of behaviours, it is easier to recognise changes that could indicate suffering. Inappropriate animal husbandry can lead to physiological and psychological stress and this may reduce the quality of the science[12].

Refinement of animal housing and care is as important for good animal welfare as it is for good science, but there are some conflicting demands and mixed messages that can make implementing this difficult. For example, there may be different interpretations of animals' behaviour, what they need and how this can be safely provided, and a lack of awareness about refinements that have already been validated and are successfully used. People may have concerns that cages could become cluttered with items that animals do not really need, wasting time and money and making it difficult to check the animals and keep the cages clean. There may be an unwillingness to risk any disruption to standardised research methods or to change established or traditional practices without proof that animals will benefit and that data quality will not be compromised, particularly if there will be difficulty in obtaining the necessary financial and human resources. In order to reconcile these issues there should be objective evaluation of what is best for the animals.

Evaluation of enrichment and other refinements to housing and care

There are three important considerations when evaluating a husbandry refinement: the potential impact on:

1. *the animals*: whether it is relevant to the needs of the species and strain; whether it could genuinely improve welfare and

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encourage a range of desirable behaviours; or whether it could cause undesirable harms, behaviours or mental states, such as toxic reactions, aggression or anxiety;

2. *the science*: whether it could have a significant impact on scientific validity;

3. *the management of the animal facility*: whether it would require changes to animal house management and resource allocation.

Dissemination of the results is also important. Feedback and communication are important, whether or not the enrichment was a success. Enrichment should be discussed in research establishments, such as in ethical or animal care and use committees, species user groups or scientific user committees and external meetings.

Evaluating the effect of a potential refinement on animals

When considering how to improve standard laboratory animal housing it is important to think in terms of the senses and behaviour of the animal species (and strain) that the housing will accommodate. For example, mice and rats are nocturnal 'prey' species that live in an olfactory and tactile world; they can communicate in ultrasound, are sensitive to ultraviolet light, live in social groups and have large home ranges. Unsupplemented and unmodified commercial laboratory rodent housing does little to meet the animals' needs, although it does meet human requirements for uniformity, hygiene, disease control, economic viability and efficient use of space.

It is highly desirable to reduce the conflict between the needs of humans and animals by defining housing that will better fit the animals' requirements. A good foundation for this is reviewing the literature on the natural behaviour of the species and the environment that it evolved to inhabit. Animal welfare science research techniques can then be used to evaluate whether animals benefit from particular refinements and, if they do, how much they want them.

Sometimes it is argued that years of domestication and inbreeding have changed laboratory rodents, especially rats and mice, so that they no longer need many of the environmental features that are important to animals in the wild. This is not the case; rodents undoubtedly can adapt to their living conditions, as can any species, but this does not mean that the similarities between rodents in the laboratory and their wild counterparts should be ignored. Many studies where laboratory mice and rats (even of highly inbred strains) and cage- farmed rabbits have been released into large, outdoor enclosures have shown that complex, natural behaviours are innate, even after many generations of domestication. Social behaviours, dominance hierarchies, tunnel- and nest-building behaviour and anti-predator behaviours are all rapidly expressed (see www.ratlife.org). Standard mouse caging represents under 3% of the 2 m^2 range observed in some wild mice. A standard size guinea pig cage is just 0.03% of the wild animal's 1500 m^2 range.

Asking animals what they want

Animal welfare science is a very broad discipline that studies animal physiology and behaviour at a variety of levels, from measuring the level of 'stress' hormones in the blood, through to studies of the behaviour of whole animals and the way that they interact with other animals and the environment. There are three broad areas of study that are often used when evaluating refinements: monitoring behaviour, choice or preference tests and motivation tests.

Monitoring behaviour

Animal behaviour is relatively easy to observe and monitor, but it is difficult to interpret and define which behaviours are desirable in a captive situation. Animals should be free to display a range of natural behaviours, appropriate for their species, but of course not all 'natural' behaviours are enjoyable (such as fleeing from a predator). Before monitoring behaviour it is essential to be able to define which behaviours are 'normal' and 'desirable'. In practice, the aim is generally for animals to display a wide range of behaviours as appropriate for the species, but advice should be sought on defining and interpreting animal behaviours, because it may not always be straightforward which are *desirable* in a captive situation. Distinctly abnormal behaviours are easier to identify. For example, it is known that a stereotypic behaviour indicates that an animal is (or has been) struggling to cope with an inappropriate environment. The behaviour may be 'normal' in a given situation, but is not desirable. The goal with laboratory animal housing should be to protect animals while allowing them to carry out appropriate occupations such as exercise, foraging, nesting and social interactions including play.

Animal behaviour should be recognised and monitored as objectively as possible. One common method is to set out an ethogram, which is a list of behaviours that may be carried out by an animal, and use this to calculate a time budget, which lists the percentage of time that an animal spends on different activities. Ethograms and time budgets can be used to assess animal

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behaviour before and after an enrichment item is added, for example, to see whether and how the item is being used. A good protocol for monitoring animal behaviour in response to refinement will also include behaviours that indicate positive welfare, which could include a good level of social interaction with cage mates, play and positive interactions with human handlers. A Wiki Ethogram for the laboratory mouse is available online at www.mousebehavior.org (note US spelling).

Animals may only perform some behaviours for a brief period of time, yet the ability to do so may be very important to them. For example, certain cage features might be important during aggressive encounters, or group-housed animals may spend only short periods grooming one another. When setting an appropriate time period for use it is important to think about how the enrichment or resource is used and any suffering that might result if it were not available, even if the animal only interacts with it for a short time.

Choice or preference tests

These tests offer animals a simple choice between two or more different environments. They are based on the fact that animals generally choose in their own best interests, except in highly artificial or confusing circumstances, so there is a close connection between the conditions animals prefer and those in which their needs are met and their welfare is good. Preference tests may provide a choice between (1) a standard cage and one containing an enrichment item, (2) two types of enrichment or (3) any two environments that provide different conditions such as temperatures or light intensities. Animal preference is an important way of assessing enrichment, as the animals' choices indicates what they really 'want'. However, animals may not make good choices between artificial options, so they may make choices that are not in their long-term interests. The preference test must be designed so that the animal is familiar with all of the options and knows how to select them. Care must be taken to design preference tests correctly, and not to allow factors such as novelty or time of day to act as confounding variables. Preference tests can show which of the available items, or conditions, an animal prefers. This may provide sufficient information to make a decision; for example, on which kind of nesting material to buy or how to set light levels. However, these tests do not indicate how important it is to the animal to have the preferred option. This can be assessed by carrying out a *test of motivation*.

Motivation tests

Motivation tests involve animals choosing to 'pay a price' to gain access to something they want, such as more space, a nest box or their cage mates. The 'price' can be lifting a weighted door, squeezing through a gap or having to make the effort to climb up a slope. The slope is increased with each trial, and the point at which the animal will no longer make the effort to climb it represents the cut-off point for the price it is prepared to pay. This type of study has allowed researchers to gain considerable insights into what animals really want and need, and how the importance they attach to features of their environment can vary at different developmental stages, times of day, seasons and stages in their reproductive cycles.

Practicalities for evaluating whether enrichment works

Before any enrichment is provided, expert advice should be sought on the natural behaviour of the animal, and then evaluation studies carried out to make sure the enrichment is having the desired effect. Before evaluation studies are conducted it is essential to seek advice on experimental design and analysis. Depending on the type of study it may need to be discussed with the regulator and others who could be affected, such as the care staff and researchers who will be implementing the refinement and working with the animals involved, respectively.

When deciding on a husbandry refinement and how to evaluate it, preliminary considerations include:

- current housing, husbandry and care, and any areas where refinement is required, for example whether animals are singly housed or the environment is barren,
- what the best type of refinement might be to address any outstanding needs,
- if an enrichment item is proposed, whether it is safe and suitable,
- who the evaluation study might need to be discussed with, in-house and/or externally,
- how much time would be available for the study and when staff would be free to conduct it.

Enrichments and resources may be divided into two categories – (1) enrichment items and resources and (2) environmental conditions and husbandry refinements – due to the different approaches to evaluating each. In the case of enrichment items, monitor how an animal's behaviour changes, quantify the level of interaction with the item and see how the animal uses it. With environmental conditions and husbandry refinements, evaluate how behaviour changes and, in some cases, whether the animal chooses (or is prepared to work for) a particular environment.

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There are some important points to consider in relation to animals' potential responses to discrete enrichment items such as chew blocks. For example, patience may be required if animals are neophobic (e.g. rodents); individuals should be able to retreat from new items if they are feeling anxious and be allowed time to habituate if necessary. If there is a risk of competition for new items, these should be supplied so that each individual has easy access (e.g. refuges) or there is at least one each (for smaller items). As well as thoughtful experimental design, these issues can be addressed by ensuring that everyone responsible for monitoring the animals is familiar with any signs of harm or distress that might occur, and is aware of end points at which the study should be stopped.

For a scientifically valid assessment of the impact of a refinement there has to be a control condition, which is not subjected to the experimental manipulation but is the same (or as similar as possible) in all other respects. Only one factor should be varied at a time, otherwise it is not possible to tell which has made a difference. In the case of studies to evaluate refinement, the control condition should be animals housed and cared for according to the normal husbandry protocols at the establishment, not animals in a barren enclosure. For example, if rats are normally group-housed with nesting material and chew blocks and the study aims to evaluate the effect of a refuge, then the control condition is groups of normal size and composition, nesting material and chew blocks and the experimental condition is the same but with the refuge added. A potential confounding variable that should not be overlooked is extra human attention, as colleagues may be interested in refinement evaluation studies, but can inadvertently ruin them by going to look at the animals more frequently than would normally be the case.

Data should also be collected so that the right kind of statistical analysis can be applied, which means using the right number of animals, making the right number of observations and using the right statistical tests. It is a good idea to consult a statistician when designing studies – rather than finishing the study and then asking for advice – as it may be the case that the data cannot be analysed and the whole study would have been pointless.

If the trial indicates that the refinement would benefit the animals, the next stage is clearly to consider how to implement it. It is important to introduce the new items at appropriate times and to monitor animals carefully in the short and long term. If an enrichment item is not used appropriately or as envisaged then its use should be carefully reviewed and consideration given to altering it or trying something different. Different strains can have highly specific needs and preferences, and it can take time to devise the most appropriate husbandry protocol for each one.

Evaluating the effect of a potential refinement on science

Once it has been demonstrated that a refinement will benefit animals and not cause them harm, the next step may be to address any questions about its impact on scientific validity or data quality. For example, there may be concerns that refinement will introduce another variable, which might reduce data quality; that more animals might be required due to increased variability; or that the data may not be comparable with previous data obtained from animals kept in standard housing[13].

All of these concerns need to be explored, to see whether they will occur in practice and whether this would actually be a problem. It should never be assumed that any refinement will adversely affect scientific results without *objectively* evaluating the impact, for example via literature searches or pilot studies, in which a small number of animals on the scientific study in question are provided with the husbandry refinement and the data analysed to see whether it has been affected. The use of pilot studies may raise concerns about using additional animals in regulated procedures, but it may still be possible to use the data in the main study, in which case there are no additional harms. Even if the data are not suitable, it may be appropriate to implement the refinement, while quantifying and allowing for its effects, as there will be a longer-term benefit for animals if the welfare of individuals in subsequent studies is improved. There will also likely be a scientific benefit, as different data from animals in suboptimal housing may well be flawed.

Concerns are sometimes expressed that accommodating the effects of husbandry refinements on scientific data may necessitate using more animals, which is obviously counter to the principle of Reduction. However, this needs to be assessed on a case-by-case basis, as there are many instances where it is preferable to use more animals, provided that each one suffers less and has a better quality of life. It is very important to give priority to the experience of individual animals when balancing the need to reduce numbers against the need to reduce suffering. The local animal welfare and ethical review body can play a role in considering the harms and benefits of different approaches and resolving any conflict between the desires to reduce and refine animal use.

It is extremely important to promote dialogue and greater awareness of the implementation (and benefits of evaluation) of husbandry refinements, as this can help to allay any concerns about their impact on the science and also help in the interpretation of scientific publications. Adequate detail of husbandry protocols should be included in papers, posters and talks; for example, the Animal Research: Reporting *In Vivo* Experiments (ARRIVE) guidelines outline what is necessary and how this can be achieved

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(www.nc3rs.org.uk/arrive)[14].

Evaluating the effect of a potential refinement on animal house management

After establishing that animals will benefit from enrichment and the science will not be adversely affected (or may even benefit), the altered husbandry protocol will need to be resourced, with respect to funding and time, and introduced on a wider scale. Different establishments will have different processes in place for this in enrichment evaluation, but all should involve (1) risk assessment and (2) evaluating the practical and financial issues associated with the changes in husbandry.

The assessment should aim to predict, as far as possible, any risks to:

- animals: this will include risks such as injury or aggression, to ensure that these can be minimised,
- humans: such as problems with ergonomics, manual handling issues or potential for disorders such as repetitive strain injury (RSI);
- equipment: for example, whether cages or cage washers could be damaged.

The process should involve defining clear action points so that all relevant personnel know when and how to respond if any hazards occur to the animals, staff or equipment once the refinement is implemented. There should also be an effective system for recording problems in any of the three areas and reporting these to the relevant individual(s) or body.

It will also be necessary to quantify how much it will add to working time to implement the refinement; for example, regarding cage or pen cleaning and monitoring animals (during the initial implementation of the refinement and in the longer term). Additional staff resource may be necessary to accommodate the refinement, which should be offset against the animal welfare and scientific benefits that are likely to be realised. Where the husbandry refinement is in the form of an item such as a refuge, additional factors to be considered include its provenance and cost; whether it can be reused (and if so how it is cleaned and how long it will last); and whether there is a long-term supply of the item, as it would be highly undesirable to have to change it part-way through a long-term study. A pilot study may be necessary if it proves to be difficult to predict the various risks and other practical issues. Conducting this during acclimatisation or habituation periods could help to allay concerns relating to study delays.

There should be regular discussion and exchange of ideas and review involving animal care staff, veterinarians, scientists and the animal welfare and ethical review body; each individual approach will depend on a variety of factors including the nature and purpose of the studies, the size of the establishment and its organisational systems.

Supply and Transport

One aim of the EU Directive 2010/63 is to guarantee the quality of laboratory animals and to provide safeguards, ensuring that, whenever possible, the animals used have been specifically bred for the purpose. The Federation of Laboratory Animal Science Associations (FELASA) code of practice3 requires that regular health monitoring programmes be implemented in breeding establishments, to ensure that laboratory animals remain disease free. Many commercial breeders now exist which can deliver high-quality, high-health laboratory animals. Research animals may come from a wide variety of sources. They may come from the same facility in which the experiments are being carried out, from another animal facility in the same organisation (for example, another animal unit of the same University), from colleagues or collaborators at another research institute working in the same field, from a commercial supplier or even from the wild. Some of these sources may be from another country which may or may not be within the EU.

The transport of animals within the EU is regulated by EC Council Directive 91/628/EEC on the Protection of Animals during Transport[15]. The import or export of laboratory animals must comply with the relevant international legislation and transport regulations. For animals travelling by air, the International Air Transport Association (IATA) Live Animals Regulations define the responsibilities of shippers and carriers, and container requirements, and are updated annually. The needs for individual species are listed, including those covered by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). These regulations have been adopted as standard by many countries. Arrangements for the transport of animals should ensure that their well-being is not jeopardised, and that they arrive at their destination in good health. Particular attention should be paid to the health and welfare prior to shipment in order to ensure that there are no subclinical infections that could cause clinical disease during or shortly after transport. Containers should be designed to provide comfort and minimise stress to the animals. They must be leak-proof and escape-proof, be able to be disinfected and be such that the animals cannot damage themselves during transport. Sufficient space should be provided such that animals will not feel any discomfort, taking into

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consideration the conditions which will prevail during transport. Animals to be transported together should be the same age and sex and from the same source. Species should not be mixed in containers, and animals should not be transported in the same vehicle as their natural predators. There must be adequate ventilation within the container. Vents should be placed on opposite sides of the containers and in such a position that they cannot be occluded. The temperature should be within the thermoneutral zone for the animal. Bedding should provide comfort and absorb moisture, insulating the animal against temperature changes and vibrations. Rodents and rabbits need free access to food and water throughout the period of transport. Water should be available, and provided in leak-proof containers, as mash, fruit or vegetables, or as a commercially available 'solid water' gel. It is preferable for dedicated vehicles and staff with suitable experience and training to be used for transportation. Vehicles should be insulated and air-conditioned, with a ventilation system that is independent of the main engine. Alarms should warn of failures of the heating and ventilation system. The vehicle needs to be able to be cleaned and disinfected thoroughly, and back-up systems should be available in case the vehicle breaks down. Animals should be examined by a competent person as soon as they arrive, and the transport should be arranged in advance so that animals can be unboxed and placed in fresh ready-prepared cages immediately on arrival. Stress caused by transport may last for up to 5 days and affects physiological processes such as immunity and reproduction.

Aspects of Health, Safety and Security Affecting Animal Care

There are many potential hazards from research; however, the risks can be minimised by implementing simple control measures through good housekeeping. For example, surfaces should be kept clean and clear from obstructions, waste and other materials. Food bags or waste should not be stored in corridors where people may trip and floors should be left dry after cleaning. Using a vacuum when cleaning cages reduces the risk from airborne allergens. There are many potential hazards associated with research protocols, which may be related to the inherent dangerous qualities of the experimental agents, for example chemicals or virulent biological agents, or the complexity and type of the experimental operation, such as the need to create dust, dander or vapours. Having recognised and identified the hazards, it is possible to devise a protocol for minimising exposure. While the employer in the UK has overall responsibility under the UK Health and Safety at Work Act 1974 to ensure the health and safety of personnel, it is nonetheless also the responsibility of individuals to make sure that they conform to the local rules, for their own safety and that of others.

Researchers may find themselves exposed to numerous hazards in the course of their work. These hazards may be from the animals themselves, or equipment, chemicals, electricity, radiation or infectious agents found in the laboratory. The risk to which the researcher is exposed, however, depends upon a combination of the nature of the hazard and how the hazard is contained to reduce or eliminate exposure. For example, a potentially hazardous compound may present only a minimal risk to personnel if handled in a safety cabinet while wearing appropriate protective clothing. The competence and training of personnel and precautions taken to reduce exposure play an important part in controlling risks in the workplace.

In the UK, the Health and Safety at Work Act 1974 imposes a responsibility on the employer to ensure safety at work for all their employees. Employers have to ensure so far as is reasonably practicable, the health, safety and welfare of their employees at work. This responsibility includes a duty to provide a safe working environment and premises, safe systems of work and appropriate training and supervision. Employers should have written rules and procedures covering basic health and safety requirements. Occupational health and safety personnel need a detailed knowledge of legislation in order to be able to translate it into working practices. In many countries, some occupational diseases such as occupational asthma and some zoonoses must be reported to government agencies, and compensation may be awarded to personnel developing such occupational diseases. Employers are required to respond to employee risk factors, and may have to make difficult decisions about suitability for work both before and during employment. If appropriate action to protect employees is not taken, then the enforcement authorities can resort to legal action against the employer. Increasingly, insurance companies are requiring details of potential hazards and risks in the workplace, and evidence of suitable methods of control, before they will issue policies to employers.

COSHH, CLP and REACH

Control of Substances Hazardous to Health (COSHH)

COSHH is the UK law that requires employers to control substances that are hazardous to health. Workers' exposure to hazardous substances can be prevented or reduced by:

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- finding out what the health hazards are,
- deciding how to prevent harm to health (risk assessment),
- providing control measures to reduce harm to health,
- making sure they are used,
- keeping all control measures in good working order,
- providing information, instruction and training for employees and others,
- providing monitoring and health surveillance in appropriate cases,
- planning for emergencies.

Most laboratories use substances or products that are mixtures of substances, or create substances, which could cause harm to employees, contractors and other people. The COSHH Regulations apply to any substance which is listed in Table 3.2 of part 3 of Annex VI of the CLP Regulations (see www.hse.gov.uk/coshh/detail/substances.htm) and for which an indication of danger specified for the substance is very toxic, toxic, harmful, corrosive or irritant. This includes:

- any preparation (mixture) that is dangerous for supply,
- any substance which has a Workplace Exposure Limit (WEL),
- any biological agents used at work,
- any dust other than one with a WEL at a concentration in air above 10 mg/m^3 averaged over 8 h, or any such respirable dust above 4 mg/m^3 over 8 h,
- any other substance that creates a risk to health because of its properties and the way it is used or is present in the workplace.

The COSHH Regulations define good control practice as follows.

- Design and operate processes and activities to minimise emission, release and spread of substances hazardous to health.
- Take into account all relevant routes of exposure (inhalation, skin absorption and ingestion) when developing control measures.
- Control exposure by measures that are proportionate to the health risk.
- Choose the most effective and reliable control options which minimise the escape and spread of substances hazardous to health.
- Where adequate control of exposure cannot be achieved by other means, provide, in combination with other control measures, suitable personal protective equipment.
- Check and review regularly all elements of control measures for their continuing effectiveness.
- Inform and train all employees on the hazards and risks from the substances they work with and the use of control measures developed to minimise the risks.
- Ensure that the introduction of control measures does not increase the overall risk to health and safety (www.hse.gov.uk/coshh).

Classification, Labelling and Packaging (CLP) Regulation

The European Regulation (EC) no. 1272/2008 on classification, labelling and packaging of substances and mixtures – the CLP Regulation – came into force in all EU member states in 2010, replacing previous legislation. The CLP Regulation adopts the Globally Harmonised System (GHS) on the classification and labelling of chemicals and is being phased in through a transitional period which runs until 1 June 2015. The intention of the CLP Regulation is that substances and mixtures that are placed on the market should be classified, labelled and packaged appropriately and in due course that the same classifications and labelling will be used throughout the world with standardised hazard pictograms.

Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)

REACH is an EU regulation concerning the registration, evaluation, authorisation and restriction of chemicals, placing duties on businesses producing and transporting chemicals. Producers and importers have to 'register' a dossier of technical information about each substance they manufacture or import above a certain weight each year. REACH should result in more information being passed down the supply chain to users. For example, safety data sheets will give more information about the exposure scenarios and risk-management measures that should be taken when using a product.

Genetically modified organisms

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Work with genetically modified organisms (GMOs) is covered in the UK by the GMO (Contained Use) Regulations 2000. These provide for human health and safety and environmental protection from genetically modified microorganisms (GMMs) in contained use, and additionally the human health and safety from genetically modified plants and animals (GMOs). The key requirement of the GMO (Contained Use) Regulations is to assess the risks of all activities and to make sure that any necessary controls are put in place. The GMO (Contained Use) Regulations provide a framework for making these judgements, and place clear legal obligations on people who work with GMOs. These regulations require risk assessment of all activities involving GMMs and activities involving organisms other than microorganisms. A classification system, with four levels of containment, is based on the risk of the activity independent of the purpose of the activity. In the UK, notification of all premises to the Health and Safety Executive is required before they are used for genetic modification activities for the first time. Activities involving genetically modified animals and plants which are more hazardous to humans than the parental non-modified organism also require notification.

Health and safety in animal facilities

Animal facilities may present specialised hazards, which are unique. Such facilities are often isolated geographically from other areas of the institution, and the work may involve particular hazards not encountered in other parts of an establishment. It is particularly important that the safety policy for these units has been properly worked out and is familiar to all personnel involved. A set of local rules should be available in each unit, which covers both general and specific safety hazards in the area.

The hazards

The major potential hazards to animal handlers can be divided into three groups:

1. allergy,

2. infection,

3. injury.

It is likely that all animal handlers will encounter at least one of these hazards during their careers. The problems involve both occupational health and occupational safety, and it is important that the institutional occupational health advisor (physician or nurse) and safety officer liaise closely in the investigation of occupational hazards and when deciding upon appropriate control measures.

Allergy to laboratory animals

Close contact with animals and their products and fur is generally unavoidable by animal technicians and research workers. However, 10–44% of animal care workers suffer from allergic symptoms, and about 10% develop occupational asthma. Allergies can have serious consequences for individuals in terms of health and future careers. Most allergies develop within 2 years of exposure, and up to half of those affected will develop signs so severe that they need regular treatment or are obliged to stop working with animals altogether. Those with pre-existing allergies are more likely to develop animal allergies. A number of symptoms are commonly recorded in cases of allergy to laboratory animals (ALA), including rhinitis and conjunctivitis. These two symptoms are the commonest associated with animal allergy but there can also be effects on skin including urticaria, wheals and eczema, particularly on the backs of the hands. Some people are affected by asthma, allergic alveolitis or can even have anaphylactic reactions, which may be life-threatening.

Since ALA is very common, regular health surveillance of animal handlers is required. Individuals identified as having major allergies prior to employment may be advised to seek alternative employment. Otherwise, regular symptom questionnaires and self-reporting of changes to occupational health advisers should identify those with developing allergies. If symptoms continue to progress despite the control measures, individuals may be wise to consider alternative careers.

Several species are involved in ALA. The allergens in each case are different, and differences in the particle size and mode of spread lead to differences in the types of allergic symptoms noted and the appropriate control measures. The rat is the species most commonly involved in ALA. The main allergens are a protein in urine and saliva. Disturbance of rat litter leaves particles airborne for 15–35 min. In mice, the main allergen is urinary protein. Although most allergens will produce symptoms in sensitised individuals at very low levels of exposure there is some evidence that controlling levels may reduce the amount of animal allergy and its severity. The risk of developing allergy seems to be related to the duration of exposure, and exposure concentration, which is task-related: cleaning out produces more allergens than simple handling.

ALA remains a major problem despite control measures, but reducing exposure is still highly desirable. The UK COSHH

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Regulations Approved Code of Practice ideally requires elimination of the allergen, and where that is not possible a series of measures designed to reduce exposure. Animal allergens can be controlled by adequate general ventilation, specific ventilation for animal rooms, and especially designed cleaning systems to reduce the likelihood of aerosol production. Personal protective equipment (PPE) is essential for all people working with laboratory animals or entering the facilities and correct disposal or laundering of this is also required. A well-designed facility with good engineering controls can also reduce the level of exposure. Airborne allergen levels depend on the rate of production and the rate of removal. Production depends on the number of animals, removal depends on the ventilation. For rat allergens, increasing relative humidity can reduce the airborne allergen concentration. High allergen exposure areas include animal holding areas, cleaning-out and cage-changing areas and non-animal areas adjacent to animal areas, for example laboratories, offices and recreation areas. The allergen exposure levels in these areas can be reduced significantly by the use of purpose-designed equipment, and particular types of bedding can reduce exposure. In animal holding areas open racking is the traditional type of animal accommodation but offers little inherent protection from allergens. Particular types of caging can reduce exposure, such as IVC racks, where each cage has a filter top to prevent exposure of staff to allergens, and when in the racking each cage has its own filtered air supply, preventing cross contamination. Cages are changed and procedures carried out in a dedicated laminar air-flow cabinet designed to protect both animals and staff. Mobile ventilated cleaning-out stations draw allergens away from staff and reduce exposure and laminar air-flow cabinets offer total containment of allergens.

Workers need to know the risks and take proper measures to control and avoid exposure. They must keep to approved systems of work which minimise their exposure to allergens. Frequent hand washing and showering on leaving the unit can help to control allergies. Air showers, which involve a blast of high-efficiency particulate air (HEPA)-filtered air, can prevent or reduce the spread of allergens to non-animal areas. These have the advantage that staff can enter in groups and no changing of clothes is required.

If engineering controls are unable to reduce allergen exposure to acceptably low levels, respiratory protective equipment (RPE) will be required. Such equipment should be carefully chosen and comply with national standards, and individuals should receive training on fitting and using it. Use should be made of coats, shoe covers, and face masks, and those who are particularly allergic may require air-fed visors or helmets with high-efficiency filtration. However, for most individuals disposable dust masks, which must be discarded after usually 4–8 h of use, will give sufficient protection to control symptoms. Simple surgical masks, however, are inadequate.

The combination of appropriate control measures and adequate, specific and sensitive health surveillance should enable most workers affected by animal allergy to continue working. There will always be those who should be advised to avoid further exposure. Careful discussion will be required on a case-by-case basis with the occupational health adviser, and redeployment of those severely affected should be arranged.

Infection

There are many well-known hazards from microorganisms and parasites normally affecting animals which can be transferred to humans. These range from the common gastrointestinal infections such as *Campylobacter* and *Salmonella*, to rare but life-threatening diseases such as haemorrhagic fevers (Marburg and Hantaan viruses). More than 150 of these zoonotic diseases have been recognised, with varying significance to humans. Modern research also produces new infection hazards, associated with emerging and re-emerging pathogens. Work with human viruses, viral vectors being used in gene therapies or transgenic animals containing proviral DNA or receptors for human pathogens could all be potential hazards.

Zoonoses

Hookworm infestation among the barefoot farmers of the developing countries is probably the commonest occupationally acquired disease in the world, and ringworm is a common zoonosis among farmers and those working with large animals, but there is little scientific data on the incidence of zoonoses in animal handlers in a research environment. Researchers working in the field may be exposed to unknown and potentially life-threatening hazards from wild animals such as Lassa fever or Hantaan virus from rodents, or filovirus infections (Ebola and Marburg) from wild primates in parts of Africa or the Far East. In such circumstances it is vital for researchers to adhere strictly to safety precautions, such as the use of protective clothing and chemical restraint, and to ensure that all animals are examined by a veterinarian and screened for zoonotic diseases wherever possible.

The risk of infection from research animals can be minimised by always obtaining animals with known health status from accredited sources and implementing regular health screening programmes to ensure their health is maintained, particularly where a COSHH assessment has identified a risk of zoonotic infection. Where health screening is not possible, for example with wild animals, they should be treated as though in quarantine. It is also essential to use suitable handling techniques to avoid bites and

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scratches.

Particular hazards from simians

The most dangerous zoonotic infections that researchers are likely to contact are those which are carried by non-human primates. Simians carry a number of particularly pathogenic diseases, ranging from salmonellosis to tuberculosis, and the UK Medical Research Council has made particular recommendations with respect to simian herpes virus (herpesvirus simiae, B-virus) and simian retroviruses.

Working with pathogens

Many research projects involve the deliberate inoculation of animals with known pathogens, or working with tissues or body fluids. All such work should be considered potentially hazardous, and a COSHH assessment performed. Local rules can then be drawn up detailing methods for safe handling and disposal of the material. Material may be screened prior to inoculation to ensure no pathogens are present, but if this is not possible assume the material is infected. Human blood and tissues should be screened for hepatitis B, hepatitis C and immunodeficiency viruses as a minimum. Animal tissues may also have the potential for carrying pathogens.

Appropriate containment levels are mandatory for work involving introduced pathogens, and flexible film isolators, individually air-filtered ventilated cages or filter-topped cages may be used. Biohazard containment levels are determined by the severity of the disease caused to man, the mode of transmission, the availability of prophylaxis or therapy and the risks of handling and caring for infected animals.

To help prevent biological hazards:

- avoid the use of sharps and take great care,
- keep hands away from mouth, nose and eyes,
- wear protective gloves and coat,
- remove gloves and wash hands before leaving animal areas,
- use mechanical pipettes (not mouth pipettes),
- never eat, drink, smoke, handle contact lenses or take or apply medicines in animal areas,
- take care to avoid creating splashes or aerosols in animal areas,
- if creating aerosols, use a safety cabinet,
- wear eye protection,
- keep animal-room doors closed,
- decontaminate work surfaces promptly and wipe up spills,
- decontaminate infected waste before disposal,
- use secondary leakproof containers to transport samples.

In some cases vaccination of the handler will be needed, for example those working with hepatitis B, but this this should be done on a case-by-case basis in consultation with the occupational health adviser.

Health surveillance

It can be difficult to identify individuals affected by microorganisms encountered in the course of their work. It may be necessary to consider taking pre-employment serum samples for storage, particularly from people working with potentially life-threatening human viruses such as human immunodeficiency virus (HIV) or hepatitis B, in order to rule out prior exposure. In any case, it is usual to take samples for storage from anyone who sustains a percutaneous injury involving contaminated blood or tissues.

Injury

Most animal species, vertebrate and invertebrate, will react defensively if assaulted, and this can pose a hazard for the researcher. Handlers may sustain a variety of injuries from animals including traumatic injuries, bites and stings. Farm animals by their sheer bulk can unintentionally cause considerable injuries including fractures, simply by treading on a foot, kicking or crushing. Smaller species may scratch. Injury may also be caused by lifting heavy animals and equipment, or twisting rapidly to catch a mouse escaping at high speed. Injuries may vary from very mild scratches or bruises to life-threatening physical injuries. Bites may be non-toxic, for example from pigs, dogs and small mammals. Even if the animal is a high-health laboratory-bred mouse, bites can become infected with a variety of commensal pathogens including streptococci and staphylococci which can be dangerous for

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humans. From animals such as snakes, lizard and spiders, bites may contain toxins and it is essential to have rapid access to appropriate medical advice in case of such injuries. Stings may result from handling fish such as weavers, stone fish, scorpion fish, etc., coelenterates, and echinoderms (starfish and sea-urchins), mollusca (cone shells) and arthropods (including bees, wasps, hornets and scorpions).

The prevention of injuries is difficult due to the unpredictable nature of many species. However, many injuries are preventable by good handling, and by taking into account factors that might trigger a traumatic event. For example, sudden noises or unfamiliar personnel may frighten an animal and cause it to react unpredictably. It is important to be aware of the animal's biology and behaviour in reducing the risk. The animal handler must understand the species they are working with, be aware of their habits and likely defensive tactics, and be trained to approach the species correctly. Strains of animals vary in temperament, and consideration should be given to selecting animals with placid temperaments. Specifically designed containment and caging facilities are required particularly with large animals when specimens are to be obtained. In the wild, anaesthetic dart techniques may be necessary to approach the animals safely.

Some species pose particular hazards. Non-human primates are strong, dextrous, intelligent and tenacious. They can pull on loose hair or neckties, and may throw things. The male of some species may be more aggressive than the female, but all species with young will react defensively to protect their offspring. Snakes and insects may bite, particularly when animals are surprised or disturbed by loud noises. Careless handling techniques without appropriate protective equipment can lead to injury.

Personnel are often unaware of the hazards associated with animal bites, such as infection, zoonotic diseases or contamination that could cause disease or be spread to others. Medical attention should be sought, and the status of the animal checked for possible zoonoses. First aid facilities should be available in all animal handling premises and individuals trained in first aid techniques to the appropriate national standard are required. They should have particular information about the nature of the animals and the likely injuries they could cause and, in the case of venomous species, particularly snakes, they will need training in specific first aid methods including standard procedures for containing the spread of venom. This may, for instance, include arterial tourniquet methods, which are inappropriate in other forms of first aid. Clear lines of communication to expertise via the local emergency facility are also required.

Miscellaneous health and safety hazards and risks

Animal handlers are exposed to a wide variety of other occupational hazards and risks similar to those which occur elsewhere. Wet floors in animal rooms can lead to slips and trips, and dim lighting or fixed light cycles may mean personnel are working in reduced lighting. The use of flammable solvents, and pressure vessels such as autoclaves and gas cylinders in the laboratory pose additional hazards.

Chemical hazards

Many chemicals are encountered in the laboratory, which may be flammable, corrosive, reactive, explosive or toxic, for example allergens, carcinogens, mutagens, teratogens or nephrotoxins. For most general laboratory chemicals, such as mineral acids, alkalis, phenol derivatives, aromatic solvents, preservatives, fixatives and pesticides, there will be adequate information to perform a COSHH assessment. For unknown agents or new drugs any information which is known about similar chemicals and data collected to date from preliminary toxicity studies can be used in risk assessment. Disinfection agents may cause irritation of skin and mucous membranes, including the respiratory tract and/or sensitisation of the skin and lungs. Appropriate handling techniques are described by the manufacturers and should be available. Chemotherapeutic agents, particularly those used for large animals, may require specific antidotes to be drawn up with a second person available in order to inject them rapidly. Other drugs may cause sensitisation if handled carelessly (e.g. prostaglandins) and some are carcinogenic (e.g. xylene), and will require training in special handling and containment techniques. Some volatile anaesthetics have been associated with abortion, cancer or liver disease.

Electrical hazards

Electrical hazards are ubiquitous in virtually all workplaces. Controls may be engineering controls such as circuit breakers, or via standard operating procedures. Constant vigilance is needed to prevent accidents. Electrical apparatus should be inspected on a regular basis. Care should be taken when using sharp instruments close to a power cable, and plugs and sockets should not be contaminated with animal products, or allowed to get wet during cleaning.

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Radiation hazards

Animal workers may be exposed to radiation from equipment used to expose animals to alpha, beta, gamma, neutron or X-radiation. Alternatively, radioactive isotopes may be administered to animals and the animals themselves become a source. The main legal requirements enforced by UK Health and Safety Executive are the Ionising Radiations Regulations 1999 and the Radiation (Emergency Preparedness and Public Information) Regulations 2001. Organisations where ionising radiation is used have a Radiation Protection Adviser appointed, and this person needs to be consulted when work is to begin. Areas where work with ionising radiation is carried out may need to be designated 'controlled' or 'supervised' areas. In addition, the worker may be required to undergo regular health surveillance by an approved doctor.

Ultraviolet light, microwaves, lasers and other forms of non-ionising radiation all possess specific hazards, which require specific action to protect eyes and skin, and careful control by employers and employees. Class 4 lasers are particularly powerful and may pose a specific fire hazard as well as potentially causing damage to the eyes and skin even from reflection.

There are specific guidelines for using magnetic resonance equipment. The use of non-ionising radiation is covered by the Electro Magnetic Fields (EMF) Directive updated in 2008, which applies to work activities where workers are exposed to risks from electromagnetic fields. It places a duty on the employer to conduct a risk assessment and calculate EMF strengths, to eliminate or reduce to as low as possible the risk of exposure, and, where risk cannot be eliminated, to devise measures to reduce the risk of exposure below the exposure limit value (ELV). The employer must provide the risk assessment to a nominated person responsible for health surveillance and requires an investigation and medical examination where an employee is 'detected' as having been exposed. Records of health surveillance activities should be kept.

Noise

Animal facilities are noisy places. Some animals, including pigs and dogs, and equipment such as vacuum cleaners can produce loud noises. Chronic exposure to loud sounds can impair hearing, typically reducing sensitivity to sounds above 2000 Hz. If conversation or talking on the phone is difficult, the noise is probably too loud. This can be controlled by engineering controls, where sound production is reduced through soundproofing or equipment design, or by administrative controls to reduce exposure, such as ear protection or reducing exposure times.

Sharp instruments

Sharps injuries are common in animal handlers, partly due to the unpredictability of the animals being handled. Percutaneous injury poses the risk of blood-borne transmissible disease or local infection. Care should be taken when handling sharps and good animal handling and restraint techniques are needed. Disposal in appropriate containers is mandatory. Health and safety legislation may require containers constructed to a specific standard with subsequent disposal by special means including incineration in dedicated units. Local laboratory practice in writing ('local rules') should identify methods of handling and disposal of such instrumentation.

Waste disposal

The cleaning of cages and the disposal of animal waste is a specialised technique which needs to avoid the formation of aerosols of animal products in the environment. Such aerosols may settle only very slowly despite appropriate ventilation and will pose a hazard, particularly in terms of allergen content. Animal waste should be collected carefully, bagged in appropriate containers, labelled and disposed of according to national waste disposal regulations. It must not be treated like household waste. Carcasses and tissue specimens from animals may require double bagging in heavy gauge plastic bags of particular colour or labelling and should be collected for incineration by trained personnel. Certain material associated with animal experimentation will require autoclaving prior to disposal by an appropriate route.

Lone working

Where there are identified hazards from occupations there are particular risks in working alone. This is especially so of animal handlers, particularly in the field, with the possibility of severe injury or other potentially life-threatening hazards such as snake bites or anaphylaxis. In all cases workers must have a rapid means of communication to someone who can respond on their behalf. This is naturally much easier in a purpose-built facility in the middle of a centre of population, but may be impracticable in certain remote locations. Such workers accept the (theoretically) major risks associated with such work, but in order to make this

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judgement they must be aware of the potential risks and make their own assessment in conjunction with their employer. Some lone workers carry alarms which are activated if they change position rapidly, and the mandatory use of mobile phones should be considered. They should be sure that somebody knows where they are and not deviate from their plans, however tempting may be an alternative scenario. Checking into and out of animal facilities with security-coded entrances is now common and an added reassurance for the lone worker.

Employee security

The very real problem associated with animal rights groups worldwide poses a considerable threat to the personal security of animal handlers. Animal facilities should be especially secure and will require high levels of entrance/exit control. The building should be designed so that breaking and entering is difficult or impossible and high levels of alarm systems connected to local police facilities are indicated. Individual employees may wish their identity to remain confidential. In particular the home addresses and telephone numbers of animal handlers should be kept completely confidential in secure storage. Such employees should not carry identification which indicates that they are animal handlers unless absolutely necessary. However, animal handlers (along with many other employees) may wish to carry a card indicating the nature of their work in general terms, and inform their doctor of their occupation. This should increase the 'index of suspicion' of a doctor or health facility to whom they are unknown, without identifying them as animal workers, if taken ill, perhaps in a remote or strange location.

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Further reading

The Shape of Enrichment, www.enrichment.org

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Chapter 6

Principles of Animal Use and Experimental Design

There are a wide variety of matters that need to be considered before a scientific study using animals can begin. In addition to having the necessary infrastructure to support the project, the right animals must be selected, sourced, delivered and then prepared for use. This preparation can involve a number of steps, depending on the type of study. As a minimum, the animals' background health status will need to be taken into consideration. Maintenance of the health status will then lead on to decisions concerning the type of housing and husbandry that is appropriate in order to maintain that health status. The use of the animal in the experiment may then require specific preparation such as acclimatisation to experimental apparatus, individual identification, preparation with monitoring equipment or pre-sampling. Only when this is complete can the study begin.

Principles of Experimental Design

The merit of animal experiments depends upon the rigid adherence to principles of scientific method. By following these principles, data produced will be both reliable and reproducible. Three principles fundamental for successful experimental design are *replication*, *randomisation* and provision of appropriate *controls*. A typical experiment involves applying one or more treatments to an experimental subject, and measuring the differences between the treated subject and an untreated, or control, subject. In animal experiments, if all animals were the same, differences could be identified using a single animal per treatment. However, living organisms are complex and can be subject to wide variations in biological response due to apparently insignificant influences. Therefore it is not possible to determine with a single animal whether any difference seen is due to the treatment, or due to individual variability between animals. Therefore, it is essential to perform the treatments on an appropriate number of *replicates* in each group. Each replicate must be independent of the others, so there is no interference between animals in a treatment group. The wider the variation between animals in a treatment group, the larger the groups must be in order to detect differences between them.

The term used to describe each replicate is the *experimental unit*. This is the unit which can be independently assigned to a treatment, and may be an animal, a group of animals in a shared environment or perhaps an individual nerve cell.

Generally one or more *control* groups are needed, to which no treatment, sham treatment, placebo or treatment with known effects (a positive control) is applied. These are used to provide baseline data against which to compare the data from the experimental groups. Power analysis is used to determine the group size and statistical analyses are used to show the likelihood of differences occurring. For more information see Festing et al.1.

Minimising variation

Application of the three Rs requires that animal numbers are minimised. One way to reduce numbers is to minimise variability, meaning smaller group sizes are required. There are two types of variability that need to be taken into account: random effects (e.g. due to uncontrolled inter-individual differences) and fixed effects (e.g. sex, strain, age, diet, bedding), the latter of which can be controlled. Controlling variation is vital for the experiment to be designed efficiently and economically. The animals should be as uniform as possible by ensuring they are genetically uniform, that they are free from clinical and subclinical disease, housed under optimum environmental conditions and of uniform weight and age. Variables that may affect the outcome of the experiment include environment (temperature, noise, light, cage size, bedding, other animals in the group, water, diet), animals (species, sex, strain, age, genotype, health status, supplier, weight, level of aggression, stage in oestrus cycle), people (researchers, surgeon, animal technician, visitors) and experimental ones (route of injection, dose, surgical quality, anaesthesia, analgesia, preparation of solutions). The experimental design should, as far as possible, remove all sources of difference for the experimental unit except for the treatment or intervention under investigation. However, for biological systems involving animals this is rarely possible so

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randomisation is essential, to ensure that the inescapable differences are spread among all the treatment groups with equal probability. Animals should be distributed between treatment and control groups randomly.

Where there is a known variable, for example sex, then this can be taken into account by using blocking. Here animals are divided up into blocks according to the known variable (e.g. males and females), and then treatments applied randomly across each block. This prevents the treatments from being allocated randomly to animals exhibiting one characteristic (see Figure 6.1).

FIGURE 6.1 Diagram of a randomised block design. Animals in each block are as similar as possible and are then assigned to the four treatment groups (A, B, C or D) at random.

Block 1	A	D	C	B
Block 2	B	C	A	D
Block 3	C	A	D	B
Block 4	C	D	B	A
Block 5	B	D	A	C

There are many methods which can be used to minimise the effect of variation, through different experimental designs (blocking, factorial design) or statistical analyses such as analysis of variance (ANOVA) or covariance analysis. See Festing et al.1 for full details and explanations. Factorial designs can be used to find the relationship between such variables as treatment, sex and strain; they also use fewer animals than other designs.

It is important to avoid bias due to one group of animals having a more favourable environment than another; as far as possible the animals and samples should be coded so that staff are 'blind' to the treatment groups. Otherwise it is possible that the observer may inadvertently score the treatment group differently from the control group when there is no difference.

Selection of animals: species, strain, genetically altered animals

Appropriate choice of animals is the first critical step to a successful scientific outcome. The first decision is which species of animal to use. Applying the principles of the three Rs and considering replacement, a number of procedures can be done effectively *in vitro* on cells in culture, or on invertebrates such as the insect *Drosophila.* When reviewing the choice of species, the principal factor influencing that decision will be the species that has the lowest capacity to suffer as a result of the procedures to be carried out, and the consequential housing and husbandry, which is consistent with obtaining satisfactory scientific results. Background historical data, financial cost and expertise may also have a bearing on the choices made. Although there has been an increase in the use of zebrafish for procedures, about 82% of scientific procedures were carried out on rodents in 2010 (www.understandinganimalresearch.org.uk/the-animals/types-of-animals/).

After deciding on the species, it is then necessary to decide on which stock or strain to use. More is known about the genome of the mouse than any other species, which is one reason for its popularity as a research animal, and there are many types of genetically modified mice available that are useful models for specific disease entities.

Rodents can be supplied as an outbred stock, which are colonies of genetically undefined animals. This means that the genotype of an animal at any given locus is usually unknown. Such animals usually have good fertility, with a good growth curve and a large litter size, and so are easy to produce. They are therefore cheaper and more readily available than inbred strains. (Outbred animals are known as stocks, whereas inbred ones are known as strains.) CD1 and MF1 are commonly used outbred stocks of mouse, and the Wistar and Sprague–Dawley varieties are common outbred rat stocks. A mutant is an outbred stock carrying a mutant gene such as nude or obese. The maintenance of mutant stocks depends on whether the mutant is dominant or recessive and whether homozygous mutant animals of one or both sexes are viable and fertile. Breeding systems are more complex to produce mutants because of harmful recessive genes, which may increase perinatal death and thus reduce productivity; therefore mutants are considerably more expensive to buy. Additionally special housing requirements may be necessary to protect susceptible mutants from infection.

Most frequently, research is carried out using an inbred strain, which is produced by 20 or more consecutive generations of brother×sister mating, producing animals that are 99% homozygous. Examples of commonly used inbred strains of mice are BALB/C, C57BL/6J and DBA/2. Fewer inbred strains exist for rats than for mice, but common ones include DA, F344, Lewis and PVG. Production of inbred strains requires much more by way of planned colony management than for outbred stocks, due to inbreeding depression in inbred strains, leading to a reduction in reproductive performance, survival and general fitness characteristics. These include lower weight, lower litter size, a higher pre-weaning mortality and a tendency to be more aggressive. The effects of inbreeding depression are most noticeable in the first few generations of brother×sister mating. An F_1 hybrid is produced by mating two inbred strains.

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As knowledge of genetics increases, more and more medical conditions are seen to result from the genotype. In order to understand the workings of genes and identify the causes of disease, increasing use is being made in biomedical research of genetically altered (GA) and harmful mutant (HM) animals. The genotype of an animal describes its genetic makeup, which is determined by the genes inherited from its parents. The phenotype of an animal describes its overall physical characteristics, which depend on the genetic makeup of the animal, and the interaction of the genotype with the environment. A GA animal is one in which the genotype has been altered deliberately by the insertion or deletion of DNA, in a way that does not occur naturally through mating or recombination of genes. Methods used for genetic modification include transgenesis (insertion of additional genes from the same or another species), or targeting of specific changes in genes or chromosomes, either by deletion or inactivation of genes (knock-outs), or addition of genes (knock-ins). An HM animal is one in which the genome has been altered by a point mutation or chromosomal change which do not involve insertion or deletion of DNA. Harmful mutants may arise spontaneously (e.g. the nude mouse, which is athymic and cannot mount a specific immune response), or be induced deliberately by mutagenesis, where viruses, chemicals or radiation are used to cause random changes in the genes. The offspring of animals exposed to such mutagens show random genetic changes and chromosomal rearrangements with resulting phenotypic changes. The alkylating agent ethylnitrosurea (ENU) is a powerful mutagen used in male animals including mice and zebrafish, and large number of F_1 founder animals may be produced from a single treated male. These F_1 mice can either be analysed directly for dominant mutations, or bred further to study recessive phenotypes. With dominant mutations, a clinical phenotype may only be observed in the homozygous state, but changes of specific parameters, for example a reduction in the activity of enzymes, can often be detected in heterozygotes. Recessive mutations can only be diagnosed from clinical appearance in homozygotes.

In any population, there is a considerable amount of genetic variation. This is caused by spontaneous mutations in the genome, since natural mutations occur at a low rate of about 1 in 10 000 to 100 000 individuals. The variations in genotype result in variations in the phenotype of animals, and this sometimes confers advantages on some animals over others in particular environments. This is the basis of natural selection, in which particular characteristics give an animal an advantage over its peers, so that it is more likely to survive and reproduce. This can be used as selective inbreeding: animals that bear the desired characteristics are chosen to breed, and those that do not are discarded, and thus are created the genetically stable lines of inbred rats and mice commonly used in research. However, many characteristics are poorly heritable, being controlled by many genes, so selective inbreeding is a very slow and inefficient way to increase the frequency of a particular gene in a population. Also, there can be undesirable side effects as selecting for some genes often results in the perpetuation of other, deleterious or recessive genes, leading to genetic diseases. Many inbred strains of mice and rats carry recessive genes, and are susceptible to particular diseases or exhibit poor growth or fertility2.

Transgenesis involves the microinjection of DNA containing the genes of interest into a fertilised egg, and implanting the resulting embryo into a recipient female. The effect of the transgene may depend on the background strain of the animal, effects being seen in one strain but not others depending on the other genes present3.

Embryonic stem cell manipulation was developed to overcome some of the problems encountered with microinjection into fertilised eggs. Manipulation of ES cells can also be used to stop the function of particular gene, producing a knock-out animal, or to add in a piece of DNA to change the way a particular protein is produced or regulated, producing a knock-in animal. It is also possible to delete or add in large regions of a chromosome with many genes. There are also other types of genetic manipulation (e.g. use of morpholinos or retroviral vectors) to inactivate genes temporarily and to manipulate genes in tissue specific areas.

Breeding and maintenance for both GA and HM animals is a regulated procedure, since this is deliberately done, albeit indirectly, to produce an individual embryo whose genetic makeup may result in harm, and the potential for harm persists beyond two-thirds of the way through gestation.

The nomenclature system of names and terminology is extremely important. Definition of the genetic background of the animals important to ensure the reproducibility of data and should be included in the methodology for the experiment.

Genetic and Welfare Monitoring of GA and HM Animals

GA and HM animals need to be monitored for the purity of the genetic line, in addition to the health status of the animals; and the welfare consequences of the genetic modification should also be monitored. Following genetic modification of fertilised eggs or embryos, the offspring produced are tested to make sure that the desired genes have integrated or been affected as required. It may be possible to determine this from the phenotype: sometimes the modified genes produce obvious effects such as coat colour changes, otherwise tissue samples may need to be analysed for the genes of interest. Methods such as saliva sampling or blood sampling may be possible if the gene can be amplified from tiny amounts of DNA using techniques such as polymerase chain reaction (PCR) prior to analysis. If not and the amount of DNA needed is greater, it may be necessary to take a biopsy from the

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ear. The smallest amount of tissue required should be taken.

There are considerable concerns about the effects of the genetic modification on the welfare of animals. The integration of a transgene within an endogenous gene may affect gene function, and the technologies employed in generating transgenic and cloned animals may result in an increased incidence of fetal and neonatal losses. New gene constructs may have unexpected adverse effects on the animal, producing obvious or subtle changes in the phenotype. It is expected that GA and HM animals will be monitored carefully for adverse effects, using scoring systems4. Detailed assessment of every new transgenic animal may be impractical: it can have considerable implications in terms of staff time and resources, and interference with neonatal animals may cause the mother to reject her young. It may be preferable to use a simple set of parameters such as litter size, birth weight and activity to identify those animals which are more likely to be affected. These may then be subjected to more detailed scrutiny, either as neonates or after weaning5. The welfare of affected animals may then be improved by revising husbandry methods such as floor feeding if locomotion is affected, or barrier rearing if they are immune-compromised.

Many normal animals are used in the generation of GA or HM animals, and if the techniques used are inefficient many of these animals are wasted. Many offspring produced in the initial stages may not be transgenic, and for lines maintained in a heterozygous state there will be a proportion of animals in every litter which are not carrying the gene of interest. In order to fully implement the three Rs, efforts should be made to utilise these normal animals as much as possible and to maximise the efficiency of the techniques used to generate new lines.

Background health status

Disease prevention is a prerequisite of any animal experiment to ensure scientific quality, animal welfare and safety6. Overt or subclinical infection is likely to affect experimental results so reducing reliability and reproducibility of the research. Infectious agents have the ability to cause ill health, thus compromising the welfare of the animals. Even subclinical infection can introduce greater variation between individuals, thereby increasing the numbers of animals required to produce statistically significant results. Some animal pathogens are transmissible to humans and it is essential to safeguard health of laboratory and research staff. It is therefore essential when selecting animals that due regard is paid to monitoring their health status.

While some infectious agents are pathogenic and will induce clinical signs of disease in laboratory animals with morbidity and/or mortality, many microorganisms induce only mild disease or no illness at all, particularly in cases of endemic disease. However, these cases are still important since the experimental procedures which cause stress and immunosuppression, or environmental factors, such as transportation or changes in diet, may convert subclinical infection into clinical problems. If it is recognised that an animal is unwell but that the changes are unrelated to the procedure it will be necessary to seek veterinary advice to investigate the causes of the disease and obtain advice on treatment. Different strains of mice may show various levels of sensitivity to an infectious agent, with some strains having asymptomatic infection while others may show differing degrees of clinical signs. In general the symptoms will be more serious in immunodeficient animals. The infectious agents do not only impact on the whole animal. Many will also affect experiments conducted on isolated organs or cells. They can persist in cells, tumour lines and other biological materials for prolonged periods of time and thus affect *in vitro* experiments or can be reintroduced back into whole animals with the passage of such contaminated material in animals. Thus the detection and elimination of such agents affects each one of the three Rs of Replacement, Reduction and Refinement: eradication of infection will improve the quality of *in vitro* work, reduce the number of animals that have to be used and improve the welfare if animals used are in good health. The breeding of GA and HM animals is no different. Health monitoring should be done as for any strain of animals, indeed increased use of animals with immune deficits may be contributing to the continued existence of various rodent pathogens such as parvovirus, rotavirus, Theiler's murine encephalomyelitis virus (TMEV) and pinworms7.

Since most infections are subclinical and modifications of research results due to natural infections often occur in the absence of clinical disease, the lack of a clinical indication of the infection has only limited diagnostic value. It is prevention of infection, not just clinical disease, which is important. The presence of unwanted microorganisms and the suitability of a group of animals for a specific experiment can only be demonstrated by a comprehensive health-monitoring programme. The data generated by this programme constitute part of the experimental data and should be available for consideration in the interpretation of the test results. Thus the health monitoring information should be included in the publication of experimental results. There should be microbiological standardisation of laboratory animals to produce better and more reliable results of experiments with fewer animals. For details of the effects of rodent infections on experimental data see GV-SOLAS (the German laboratory animal science association)8, and for fish see Kent et al.9.

Sources of infection

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The most important source of infection is other laboratory animals10, often due to population density and the turnover of animals within a unit. Animals entering a unit should be of known health status and come from accredited breeders. Accompanying such animals should be a report on their microbiological status and housing. There are various terms used to describe the microbiological status of laboratory animals, which in turn indicates the type of housing in which they are maintained.

- Germ-free (axenic) animals are free of any associated life and are maintained in isolators with no contact with the outside world.
- Gnotobiotic animals have one or more associated non-pathogenic microorganisms, the identity of which is known. These animals are also maintained in isolators with no contact with the outside world.
- Specific-pathogen-free (SPF) animals are free from *specified* pathogens. The SPF designation requires that the absence of the listed pathogens is supported by regular monitoring for those pathogens. These animals are maintained in barrier units which use a combination of construction, equipment (e.g. individually ventilated cages), operating procedures (including showering facilities, the provision of sterilised food, water and bedding) and restricted access to the unit to minimise any potential contact with those specified pathogens. Sometimes these are referred to as barrier-reared animals.
- Conventional animals carry normal microbial flora which is undefined. Their housing has less strict access and operating conditions. However, they are should still be monitored for the main animal pathogens.
- Wild-caught animals have an unknown microbial flora and are likely to carry pathogens that may cause disease to both staff and other animals.

Other animals brought into the unit, particularly in large facilities with multiple users, are a common cause of health status breakdown. A well-run facility will have a standard operating procedure (SOP) that should be followed for all incoming animals in order to protect the health of those already in the unit. The supplier's health profile should be examined and should provide details of what tests were carried out, who did them and how the interpretation was arrived at. Environmental sources of contamination include water, food and bedding; all of which may therefore require sterilisation before being given to the animals. Effectiveness of water sterilisation should be checked regularly and food stored appropriately to prevent contamination from wild rodents and birds or depletion of vitamin levels or the development of moulds or toxins. Quality of the bedding must also be assured. Ventilation rates in rooms must be adequate and air filters changed regularly or there will predisposition to respiratory disease. All equipment must be clean so as not to spread infection to the animals. Examples of potential sources of contamination include such items as anaesthetic face masks and gavage cannulae. Cell lines and serum may be contaminated and should be screened before bringing them into units for use in animals. During transportation animals should be protected from infection where possible by transporting them in filter-protected cages11. Good hygiene procedures should be followed at all times in the animal unit.

Rodents, and many other species, are generally supplied with a health profile from the breeder. If the animals are to be kept only short term it may not be necessary to do any more, but if they are kept long term then continual regular screening will be necessary to ensure they have not picked up extra infections. If the animal is kept under conditions to prevent entry of disease it is important to maintain these barriers and not to bring microorganisms in to the unit.

Some animals may have predisposing factors such as a genetic variant which contribute to the development of disease. Genetic monitoring is vital to ensure that this predisposition remains a constant factor within the experimental model. The susceptibility of GA strains may be difficult to predict due to changes in the immune system. Nutritional status of the animal will also be a factor in susceptibility to disease. The single most important predisposing factor in the laboratory animal is stress, which leads to immunosuppression and the subsequent development of disease. It is therefore very important to do everything possible to reduce the stress caused to animals.

Health monitoring

Health monitoring of laboratory animals is a fundamental part of disease prevention used to determine their pathogen status and general health. Always note the date of health monitoring reports of colonies of animals, as they will only reflect the health status of a population at the time of testing.

The frequency of testing, which can vary between monthly and annually, depends on circumstances. The threat of disease – either clinical or subclinical – should be perceived as serious by all those involved in order for screening to be carried out effectively and for barriers to be maintained. The choice of agents to test for is based on pathogen significance and likelihood of interference with research and includes zoonotic diseases, subclinical or clinical diseases which may cause welfare consequences, and diseases which may interfere with or invalidate the experiment. The researcher should be aware of any infections which are

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likely to affect the research, and ensure that there is an appropriate screening programme in place to detect these infections before any problems are caused. Recommendations for health monitoring of mouse colonies may be found in publications by the Federation of Laboratory Animal Science Associations (FELASA)6 and Reuter and Dysko12.

Health monitoring in experimental units is a justifiable expense and an essential means of enhancing the reliability of the experiments. Health screening of the animals should not be a theoretical exercise and action should be taken if positive results are found. The epidemiology of the disease must be understood in order to know how frequently to test, what samples to take and how to deal with a positive result if it should be found. Advice should be sought from veterinary staff. The recommendations of FELASA6 for health monitoring in each species should be followed. The purpose of health monitoring is to supply the researcher with data on variables that might influence the outcome of an experiment. These data therefore are part of the experimental work and must be considered during the interpretation of the experimental results. The results of health monitoring should therefore be included in scientific publications. The decision about which infectious agents are acceptable and which are not is never straightforward. Many that are included on the standard list are historical and there is a need for rationalisation of the selection of agents to be screened for and those which management systems should be designed to keep out of rodent units. Since most infections are subclinical, the importance of an effective surveillance and barrier system is not always appreciated until it has been breached when short cuts are taken.

It has been argued that the move towards clean animals has reduced the 'natural' challenges to the immune system and can alter results from research previously carried out under conventional conditions and may even be detrimental to animal welfare13. However, the microbial variables are a critical influence on the research model and cannot be ignored. Exclusion is the most practical solution, which requires surveillance, diagnosis, control and management. It is important to interpret results in context, which requires an understanding of the biology of each agent, and the use of diagnostic pathology to support serological results rather than relying on serology alone.

In active health monitoring, animals are taken from the population at regular intervals. Blood is taken for serological testing for a number of viral infections and some bacterial infections and they undergo full post-mortem examination to detect any other infections. *Sentinel* animals can also be used to monitor the population. These are animals of the same species whose bacterial, parasitic and virological burden is already defined. They are housed in the same rooms as the experimental population, and some of the bedding from the cages of the experimental animals is placed into their cages at each clean out session. They will thus be exposed to the same pathogens and they can be sampled to determine their pathogen status which will reflect the status of the original experimental population. Immunodeficient animals (e.g. nude mice) do not produce sufficient amounts of antibodies to give reliable test results and are therefore not suitable for serological testing, although they may be used for bacteriological and parasitological investigations. Sentinel animals should be at least 10 weeks old and should have been housed in the unit and exposed to samples of used bedding for at least 4 weeks.

Sampling strategies

The determination of the number of animals to be screened for infection is very important and depends on the assumed rate of infection and the randomness in the sampling. There is a natural inclination to keep the sample size small on grounds of economy and availability, but it must be sufficiently large to give the required level of confidence in the results. For health screening for multiple agents which may have very different infection rates, it is impossible to ensure with 100% certainty that all agents will be detected with the same degree of precision. Routine sampling is usually based on assumed infection rates of around 40–50% in order to keep samples sizes reasonable but the limitations of this need to be recognised.

It is advisable to use two age groups of sentinels since young adults will be best for detecting recent viral infections and retired breeders give an indication of the history of the colony. The FELASA6 guidelines give advice on the frequency of testing, which can vary between monthly and annually, depending on circumstances.

Which diseases to monitor

It is not practical or cost-effective to test for all known infectious agents so testing, by necessity, has to be selective. The choice of agents to test for is based on pathogen significance and likelihood of interference with research and includes the following.

- Any zoonotic diseases should be the first priority in screening. This will enable any necessary precautions to be taken to prevent spread of disease to humans.
- The second group of diseases to look for will be those that might be present as subclinical infections but which will cause overt clinical disease when the animal is subjected to an experimental stress.
- The third group to consider are those diseases whose presence, whether clinically evident or only subclinical, will interfere

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with, and may invalidate the experiment. For example, this may be the presence of a viral infection that has no effect on the animal but elevates certain enzyme levels, the measurement of which may form part of the experimental study.

A discussion with the veterinary staff will aid in designing a sensible screening programme which will be cost-effective. Before embarking on a screening programme it is important to be prepared for the possibility of positive results, and to consider what action will need to be taken if the result shows the presence of certain infections. Action should be taken if positive results are found; otherwise it is a waste of time, money and animals not just in terms of health screening, but possibly for the whole experiment. The programme will need to be re-evaluated once the status of the colony is known, as subsequent tests may need to be done less frequently, or only certain groups of the population tested, after the initial screen. Screening can be a very costly and laborious task but this should not be used as an excuse to ignore the possibility that infections exist which may be of concern to personnel, detrimental to the animals' welfare and to the validity of the science being carried out. Monitoring laboratories should follow the principles of Good Laboratory Practice (GLP) and the FELASA guidance for the accreditation of laboratory animal diagnostic laboratories.

Biosecurity and containment for rodents

Rather than conventional shoebox cages on open shelving or racks, housing for laboratory rodents is now frequently designed in such a way as to offer microbiological containment. This may be done because it is required as a strategy for the containment of animals which may pose a risk to human safety, because they are carrying biological agents that fall into hazard groups categorised by the Advisory Committee on Dangerous Pathogens. Sometimes the principal requirement is to protect staff from animal allergens, or it may be to prevent the spread of organisms between animals in order to maintain their high health status, perhaps to allow animals of different microbiological backgrounds to be housed in close proximity.

Individually ventilated caging systems

Individually ventilated cages (IVCs) are cages with filter tops held in a specialised rack that allows each cage to receive its air supply individually. Supplied air is high-efficiency particulate air (HEPA) filtered, and each cage is isolated from the others. This avoids spread of pathogens between cages, and prevents exposure of personnel to potential allergens or hazardous substances. The containment provided by the cage system must be maintained while the cage is cleaned or the animals examined. These procedures are therefore carried out in ventilated workstations or safety cabinets. There are three classes: class I protects the operator, class II protects the animal and the operator and has an easy access open front and class III is in effect a totally sealed box.

IVCs are complex cage systems that rely on engineering to support the animals' environment. If this equipment is to fulfil its objectives thorough staff training is required, and standard operating procedures are required for IVCs and safety cabinets to ensure that the required level of containment is maintained. For more information see Chapter 5.

The IVC provides the animal with an environment that is controlled for ventilation, temperature and relative humidity. The containment provided by an IVC may be beneficial: the quality of the science may be improved by using healthier animals, enabling fewer animals to be used more effectively. However, since this type of caging makes the animal more remote from the staff and the animal technician cannot always see the animal easily, cannot hear or smell the animal, these valuable sources of information when assessing its overall condition are lost. The welfare of the animals will therefore depend even more on the qualities and skills of the staff employed.

Flexible film isolators

An isolator is like a large plastic bubble, which is sealed from the outside world. Filtered air is supplied, and equipment and food are passed in through a port which allows disinfection of the packaging prior to entry into the isolator. Isolators operate under negative or positive pressure, offering complete environmental protection against the spread of pathogens in or out of the isolator, so animals of different health status can be housed within the same room, in different isolators. They are mobile but do not make good use of available floor space. They must be alarmed in case of failure of the air supply and they can be difficult to work with.

Filter-top cages

Cages are held on an open rack, but covered with individual filters. Some containment of microorganisms is achieved with well-fitting lids, but the microenvironment in the cage is compromised as there is restricted airflow. The filter top should only be

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removed in a safety cabinet, which can make them difficult to work with.

Ventilated cabinets

These are similar to sealed cupboards with their own filtered air supply. The cabinet operates under negative pressure. Inside, filter-top cages are generally used. They are easy to install, relatively cheap and mobile but can present a risk when the cabinet doors are open or at cage changes.

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Chapter 7

Handling and Techniques

Prior Considerations

Before carrying out procedures, a considerable amount of preparation may be needed. The aim of procedures is to achieve the scientific output with minimal effect on the animal, and this requires careful planning. Scientific procedures have the potential to cause adverse effects to the animal, and it is essential to minimise any distress caused.

Whatever the procedure to be done, the first step is to check the paperwork and ensure that the necessary authorities under the UK Animals (Scientific Procedures) Act 1986 (or ASPA) have been granted. You *must* check that the project licence, personal licence and Section 2C licence authorise the procedure to be carried out in the particular room. *Only* when you have checked those should you proceed. Having checked that authority exists to perform the procedure, it is essential to keep clear records of any regulated procedures performed. These are required by the legislation, and form part of the individual researcher's training record. Records should be kept of animals on which procedures have been performed, including details of supervision and declarations of competence by the project licence holder.

With small laboratory animals there are potentially a number of practical difficulties that must be addressed. Most are small in size, and therefore gaining access to suitable sites for administering compounds or taking samples is often difficult. If access can be gained, there are constraints on injection and sample volumes and frequencies determined by the welfare of the animal. For larger animals there is some risk of injury to the operator if the animal is not cooperative or adequately restrained. These points must be considered and addressed when planning the procedure.

Training and competence of operator

It is essential to practise and become competent prior to carrying out procedures on animals. It is generally not permitted to practise on live animals, but it is possible to practise on cadavers and artificial models, which can be used for techniques such as intravenous injections and gavage in rodents. Before performing a procedure for the first time, the operator should observe others skilled in the procedure, study the anatomy of the area and then practise on models before carrying out the procedure on a live animal for the first time under supervision. Do not carry out a procedure unless you feel you are competent to carry it out correctly without causing undue stress to the animal. Tutorials on common techniques in small animals can be viewed at Procedures with Care (www.procedureswithcare.org.uk) and the Norwegian Reference Centre for Laboratory Animal Science and Alternatives website (http://film.oslovet.veths.no/); see also reference[1].

Acclimatisation

Animals should be allowed to acclimatise to the environment before procedures, and this may take 1–2 weeks. See Chapter 9 for more details on acclimatisation.

Training of animals

Every day-to-day interaction between a researcher or technician and an animal results in the animal being trained to an extent, whether the person is aware of it or not[2], and the behaviour of the animal may be modified as a result. Good results can be obtained by deliberately encouraging the animals to behave in a particular way by managing this training process actively. Many animals can be trained to accept the handling required for procedures, or to cooperate actively by entering restraint devices or presenting themselves for injection. Training can be achieved through positive or negative reinforcement. Positive reinforcement training works by rewarding a desired behaviour, and is a very effective and welfare-friendly method of encouraging animals to

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cooperate. There is no need to deprive the animal of food or water with this method: animals are simply rewarded for an appropriate response. This is often used in dogs and primates, but can also be used successfully in farm animals, especially pigs, and smaller animals. In rodents, rabbits and birds, training may be basic, focusing on developing a positive human–animal relationship, but is still of benefit. More details on positive reinforcement training methods can be found in Laule2. Clicker training is a method of positive reinforcement in which a clicker is used to provide the positive reinforcement. In the training period, desired behaviour is rewarded with a food reward, and at the same time a clicker is used. In time, the animals become conditioned to the noise and will perform the desired behaviour when they hear the click. See www.clickertraining.com for details.

Negative reinforcement requires the animal to escape from an unpleasant stimulus by performing the desired behaviour. This inherently causes distress to the animals and therefore should only be used when positive reinforcement is not possible. In addition, studies have shown that positive reinforcement training methods are often more effective than negative reinforcement training[3]. Traditionally laboratory animal husbandry methods have used negative reinforcement to force animals to cooperate, for example by use of nets, boards or crush-back cages to force animals to move into a particular space. These methods can cause fear and stress in addition to the stress caused by the resultant procedure. If animals can be encouraged by reward to present themselves for procedures they can become desensitised to the handling and restraint, reducing the fear and stress caused.

Restraint of animals

Good animal handling is essential to reduce the risk of injury from bites and scratches, and minimise stress to the animals. All animals will respond to the presence of a human, and most species can recognise individuals and will be nervous of strangers. It is important for the researcher to establish a friendly relationship with the animal to reduce nervousness on both sides. An animal that is confident and relaxed with its handler will be more cooperative, enabling procedures to be carried out more easily. A brief description of appropriate handling techniques can be found in the species chapters, but with each it is essential to get advice and assistance from people with previous experience. Some animals can simply be held in one hand and the injection given with the other; for example, intraperitoneal injections in mice. For others, an assistant or a restraining device may be needed to hold the animal. For some animals, administration can be facilitated by wrapping them in a towel, such as with rabbits. If chemical restraint is needed, then knowledge of anaesthetic or sedative drugs is required (see Chapter 9).

Equipment

Various instruments and pieces of equipment will be required for procedures, whether needles and syringes, surgical instruments, anaesthetic equipment, sample pots, imaging equipment or restraint devices. Before starting the procedure it is important to ensure that all the equipment is ready and that the operator is competent in its use. Advice may be needed before selecting equipment appropriate for the procedure to be performed.

Administration of Substances

ADME

ADME is an acronym used in pharmacology for **a**bsorption, **d**istribution, **m**etabolism and **e**xcretion, and describes how a drug behaves after administration. These four factors determine the level of drug reached in tissues and rate of excretion, influencing the pharmacological activity of the compound. These factors that affect the absorption and distribution of administered compounds define the *pharmacokinetics* of the compound, and should be studied prior to administration to ensure that the compound is being given in the most effective way.

The *pharmacodynamics* of a compound can be defined as what the compound does to the body, and describe its physiological and biochemical effects, and the mechanism of action, including the relationship between drug concentration and effect[4]. This should also be considered prior to administration.

Absorption

Substances may be administered to animals topically, orally (per os, p.o.), by intravenous (i.v.), intramuscular (i.m.), intradermal (i.d.), intraperitoneal (i.p.) or subcutaneous (s.c.) injection, by inhalation, per rectum (p.r.), or by injection directly into other

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body parts such as joints or the gastrointestinal system. After administration, compounds are usually absorbed into the bloodstream before being taken up by the target cells. The rate at which compounds are absorbed into the bloodstream depends on the blood flow to the site of administration, the nature of the compound and its solubility in the tissue fluids, and the manner in which it is presented. Absorption determines the compound's *bioavailability*, which is the fraction of the administered compound that reaches the systemic circulation.

Compounds given by intravenous administration reach a high blood concentration immediately, and generally have a bioavailability of 100%. This then tails off rapidly as the compound is eliminated. Compounds given by other routes are absorbed at slower rates, Muscles have a good blood supply, so substances administered intramuscularly are absorbed more quickly than those given subcutaneously, as the subcutis has a poorer blood supply. Compounds that are highly soluble in the tissue fluids will be absorbed quickly. Some injectable preparations are designed to have a long duration of action, and the active compound in these is mixed with a carrier of low solubility to slow down absorption into the bloodstream. With orally administered compounds, absorption tends to be slower, and takes place over a longer period. However, even if the bioavailability is high, it does not necessarily mean that the blood concentration reaches the peak seen immediately after an intravenous dose. Many compounds are absorbed very slowly after oral administration, and may never reach high levels in the blood.

Compounds given orally may be absorbed at various points in the gastrointestinal tract. The presence of food, gastric emptying time, intestinal transit time, chemical instability in the stomach and inability to cross the intestinal wall all affect absorption from this route. Substances are only absorbed across the gut wall if they are lipid soluble. Absorption may be affected by pH. Compounds that are ionised are not lipid-soluble and will only be absorbed if a specific carrier exists to transport them across the gut wall into the blood. Basic or alkaline compounds are likely to be fully ionised in the acid environment of the stomach, and will be poorly absorbed. However, once in the small intestine, the higher pH will reduce the level of ionisation and increase the absorption. The reverse is true for acidic compounds. For solid compounds, the particle size affects the rate of absorption, smaller particles being absorbed more quickly.

All substances absorbed in the stomach or intestine are absorbed into the portal circulation and have to pass through the liver before reaching the systemic circulation. They may be subject to the *first-pass effect*, in which liver metabolism of some or all of the drug reduces the amount reaching the systemic circulation. Many compounds induce the liver to produce enzymes, which metabolise them, thereby increasing the first-pass effect with repeated dosing. Alternatively, there may be some *enterohepatic recycling*, in which the compound is conjugated and secreted into bile, and therefore passed back into the intestine without reaching the systemic circulation.

Some compounds are designed to be given orally and absorbed locally in the large bowel, and are insoluble unless they are activated by enzymes during passage through the stomach and small intestines. Other compounds are designed to be given per rectum. These may not be subject to the first-pass effect.

Distribution

Compounds are usually carried to the effector site via the bloodstream. After entry into the systemic circulation compounds distribute into different tissues and organs, often to different extents in different organs, and as distribution occurs the plasma concentration tends to reduce. Where and how compounds are distributed within the body depends on the blood flow to the site, the solubility of the compound in the tissue fluids, molecular size, polarity and binding to serum proteins. There are some special cases, however. For example, compounds will only enter the central nervous system if they can cross the blood–brain barrier. Lipid-soluble compounds will accumulate in fatty tissues, even if the blood supply is poor. Drugs will also tend to accumulate at sites of metabolism or excretion, such as the liver and kidneys. In pregnant animals the fetus is separated from the mother by the placenta so only certain compounds will enter the fetus, although some compounds may accumulate in the fetus. Compounds may be secreted into milk if they can cross the lipid membrane in the mammary gland. Milk is slightly more acidic than blood, so basic compounds tend to accumulate in milk because they ionise after having crossed the lipid barrier and cannot then return to the blood.

Metabolism

Compounds begin to break down as soon as they enter the body. *Increasing* the rate of metabolism of a drug *decreases* the duration and intensity of the drug's action, and vice versa.

All tissues can metabolise compounds to some extent, for example the epithelium of the gut, lungs, kidneys and skin, but the majority of drug metabolism is carried out in the liver, as it is large in volume and has a high concentration of drug-metabolising enzymes, the most important being the cytochrome P450 mono-oxygenase system. As metabolism occurs, the initial compound

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is broken down into one or more metabolites. Some metabolites are inert, whereas others may be pharmacologically active. Drug metabolism can be affected by plasma concentration, age, strain, sex, nutritional status, intestinal flora, liver kidney or heart disease. It can also be affected by concurrent administration of other compounds metabolised by the same enzymes, or induction of liver enzymes.

Excretion

Compounds and their metabolites are usually excreted by the kidneys, by biliary excretion followed by elimination in faeces, or via the lungs. The main route of excretion is via the kidneys. Compounds or their metabolites can be filtered passively in the kidney, or actively secreted into the urine by transporters.

Administration volumes

The volume of any substance given must be as small as is practicable for the procedure, and will be limited ultimately by the size of the animal. Table 7.1 gives suggested volumes for common routes of administration.

Table 7.1 Administration volumes for laboratory species.

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Species	Reference weight	Intravenous or intra-arterial (ml)	Intraperitoneal (ml)	Intramuscular (ml/site)	Subcutaneous (ml/site)	Oral (ml)	Intradermal (ml/site)
Mouse	20 g	0.2	1–2	0.05	0.5*	0.4	100
Rat	250 g	1	2–4	0.1	1–2*	5	100
Hamster	120 g	0.3	2–3	0.1	0.5–1*	3	100
Guinea pig	500 g	1	5–7	0.1	1–2*	5	100
Rabbit	2 kg	4	10–15	0.25	1–3*	10	100
Ferret	750 g	1–3	5–10	0.2	1–2*	5	–
Cat	3 kg	2–5	15–30	0.5	5–15*	3	–
Dog	12 kg†	10–15	60–120	2	15–30*	50	100
Fowl	2.5 kg	3–5	10–15	0.25	1–2	–	–
Rhesus	5 kg	5–10	30–60	1	5–10*	5–20	100

Note: figures have been calculated for the 'average' size animal for that species, for ease of reference. Adjust as necessary for the actual weight of the individual.
*Maximum of four sites.
†Beagle.

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Injections and blood samples should be performed using aseptic techniques. New, sterile needles should be used for each animal, and the injection site may need to be cleaned with antiseptic solution prior to administration. This is particularly important for intravenous administration. It is important to use equipment appropriate for the species (see Table 7.2), and use the smallest gauge needle possible. This is determined by the size of the animal and the viscosity of the substance, since thick, viscous liquids may not pass through narrow-gauge needles.

Table 7.2 (a) Suggested hypodermic needle sizes for laboratory animals*. (b) Recommended cannula sizes for laboratory animals.

(a)

Species	Intraperitoneal	Intramuscular	Intravenous	Subcutaneous
Mouse	27	29–30	27–28	25
Rat	23–25	25	25–27	25
Hamster	23–25	25	25–27	25
Gerbil	27	27	27–28	25
Guinea pig	21–25	25	25–27	23–25
Rabbit	21–23	23–25	23–25	21–25
Ferret	21–23	23–25	21–25	21–23
Cat	21–23	23	21–25	21–23
Dog	21–23	21–23	21–23	21–23
Rhesus	21–23	23–25	21–25	21–25
Sheep	19–21	21	19–21	19–21

*Needle and cannula sizes are in standard gauges. Note that different lengths are available for all gauges.

(b)

Species	Site	Gauge	Length
Rats	Tail vein	24–25	12–19 mm (1/2–3/4 in.)
Rabbit	Ear vein	24	19 mm (3/4 in.)
Dog	Cephalic or jugular vein	20–21*	25–40 mm (1–11/2 in.)
Cat	Cephalic or jugular vein	22–23	25 mm (1 in.)
Ferret	Cephalic vein	24	19 mm (3/4 in.)
Rhesus	Cephalic or saphenous vein	21–24	19–25 mm (3/4–1 in.)
Pig	Ear vein	21–23	25–40 mm (1–11/2 in.)
Sheep/goat	Jugular vein	19–21	40 mm (11/2 in.)
Cattle/horses	Jugular vein	19–21	40 mm (11/2 in.)

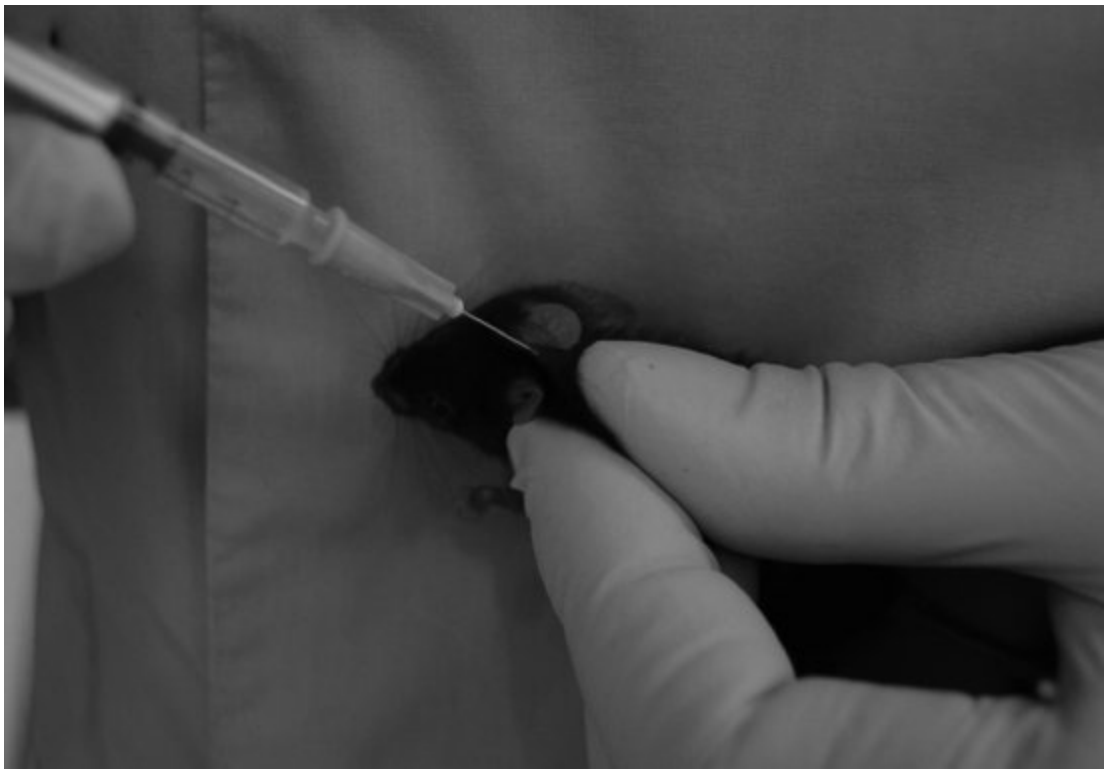
*Depends on breed and age. For puppies, a 23 or 25 G cannula can be used.

Subcutaneous injections

For most species, subcutaneous injections can be given into the scruff of the neck (Figure 7.1). A fold of skin is lifted using the thumb and first two fingers of one hand, and the needle is passed through the skin at the base of the fold parallel to the body, to avoid penetrating deeper tissues. Subcutaneous injections are rarely painful, unless the substance being injected causes stinging.

FIGURE 7.1 Subcutaneous injection in a mouse. Photo: James and Steve.

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In some animals, subcutaneous injections can also be given over the flank. The animal is restrained as for intraperitoneal injection, and the needle inserted under the skin in the middle of the flank (Figure 7.2). This site is sometimes used for injection of tumour cells, since the growing tumour can easily be assessed in this site, or for antibody production in rabbits.

FIGURE 7.2 Subcutaneous injection in the flank. Photo: James and Steve.



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In large animals, fluid can be given under the skin over the ribs. For pigs, small volumes can be injected into the skin behind the ear, or into the fold between the leg and the abdomen. Pigs have much subcutaneous fat, and injections given elsewhere are likely to enter the fat, where absorption is likely to be slow.

Intramuscular injections

Intramuscular injections are frequently painful, due to the distension of muscle fibres, and an alternative route should be used if possible. If not, good restraint or anaesthesia is required. In small animals they are usually given into the muscles of the thigh, and larger volumes should be injected into the quadriceps group on the front of the thigh1. The muscle can be immobilised with one hand while injecting with the other. In rats, the quadriceps can be palpated on the front of the thigh, and immobilised with the thumb and forefinger of one hand while injecting with the other (Figure 7.3). Injections can be given into the caudal thigh muscles, but as the sciatic nerve runs through these muscles care must be taken here or damage may be caused to the nerve. The risk can be minimised by inserting the needle into the muscle mass from the lateral aspect and angling the point caudally.

FIGURE 7.3 Intramuscular injection (quadriceps).



In mice, because the muscle masses are so small, a small needle (29 or 30 G) is required and only a small volume can be injected. Injections can also be made into the anterior tibial muscle on the anterolateral aspect of the lower hind limb. The mouse is held on its back with the leg pulled forward. It is important that the tibial tuberosity, a ridge on the front of the leg, is vertical. The needle is inserted at a 45° angle about 3 mm lateral to the tuberosity, parallel to the line of the leg. The needle is inserted to a

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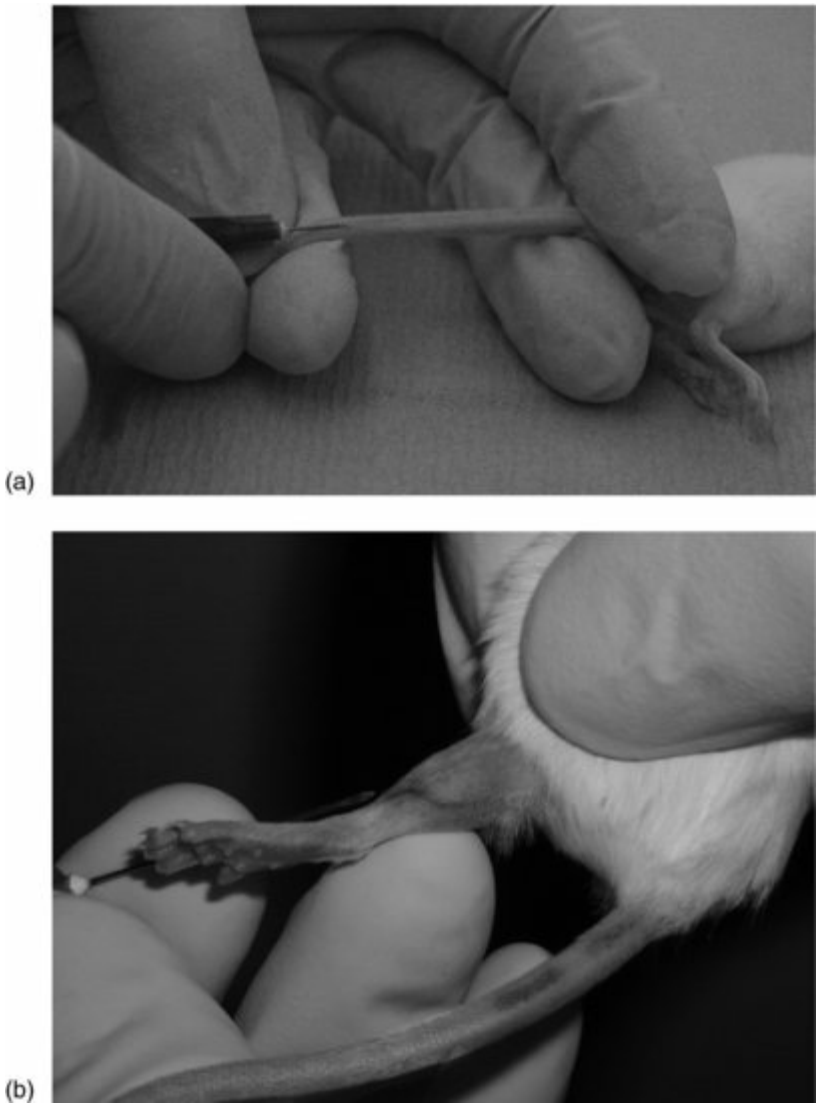
depth of 2–3 mm. Up to 50 µl can be injected[5].

In dogs, cats, ferrets and rabbits, injections can be given with care into the muscles on each side of the spine, and in large animals the gluteal muscles over the rump are used. In adult pigs, injections are given into the neck muscles, but a long needle is required to penetrate the fat layer. Piglets can be injected by lifting them by one hindleg and injecting into the caudal thigh muscle on that side. Fowl are given intramuscular injections into the pectoral muscles. After the injection, the site should be massaged to disperse the dose.

Intravenous injection

In rats and mice, intravenous injections are usually given into the lateral tail veins (Figure 7.4a). Injection is facilitated by warming the animal in a box at 37°C or placing the tail in warm water before injection. Local or general anaesthesia may be needed. The animal should be restrained by an assistant or placed in a restraining device, and the vein can be raised by gently occluding it at the tail base. The needle should be aligned parallel to the vein pointing towards the body with the bevel uppermost before sliding it gently into the vein. There should be no resistance to injection. This method can be seen at www.procedureswithcare.org.uk. In small animals injections can also be given into the saphenous vein (Figure 7.4b). The hindlimb is held in extension, and the vein raised by occluding it at the stifle joint. This is particularly useful for tail-less rodents.

FIGURE 7.4 (a) Tail vein injection in the rodent. (b) Saphenous vein. Photos: (a) MRC Harwell; (b) AstraZeneca, used with permission.



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In dogs, cats, primates and ferrets the cephalic vein on the anterior aspect of the forelimb can be used. The vein is raised by placing the first two fingers of one hand behind the elbow and applying pressure with the thumb just below the anterior aspect of the elbow: in ferrets, a tourniquet is best for this purpose as the limbs are short (see Figure 7.5). The jugular vein can be used in dogs, cats, hamsters and ferrets (see Figure 7.6), and this is the method of choice in ruminants and horses. The ear veins are used in guinea pigs and pigs. Fowl can be injected via the brachial vein.

FIGURE 7.5 (a) Quick-release tourniquet. (b) Quick release tourniquet in use for cephalic venepuncture in a macaque.

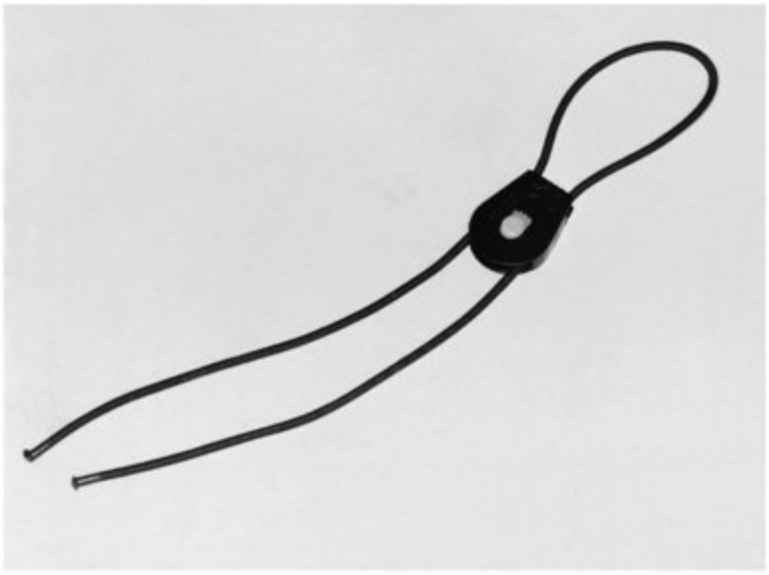




FIGURE 7.6 Jugular venipuncture in a ferret. Note the needle has been bent at an angle to the hub to facilitate location in the vein.

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If fluids are to be administered by infusion, the flow rate should be as low as possible, and the infusion given over as short a time as possible. Otherwise, if too much fluid is given too rapidly, the circulation may become overloaded, causing pulmonary oedema. The maintenance requirement for fluid is approximately 50–60 ml/kg per 24 h in normal mammals and care should be taken not to exceed this unless there are deficiencies to address.

Intradermal injections

Intradermal injections for most species can be given in the same area as subcutaneous injections. However, the dermis of most laboratory animals is very thin, and intradermal administration may require very careful needle placement under anaesthesia or sedation. The site should be clipped to remove fur and cleaned with antiseptic solution to facilitate visualisation of the injection site. The needle should be inserted bevel uppermost a short distance into the skin and the injection given. There will be resistance to injection, and successful administration should result in a raised bleb. The volume which can be injected in this site is small.

Footpad and tail-base injections

The footpad is a commonly used injection site for studying immunological phenomena in rodents. Footpad injection is a combination of intradermal and subcutaneous injection. After injection there is a delayed-type hypersensitivity reaction, which can be used to measure immune responses *in vivo*. The lymph drainage from footpad injections goes firstly to the popliteal lymph node[6], and then to the medial iliac[7] and inguinal nodes[8]. Lymphocytes can be harvested from these nodes for study *in vitro*. This method of administration is also used in the popliteal lymph node assay, to distinguish between immunostimulating and inert chemicals[9,10]. Foot-pad immunisation followed by boosting at the base of the tail is also a commonly used protocol for production of antibodies, or for induction of arthritis. However, footpad injection results in swelling and pain and can impair mobility. A more humane alternative to this method is to immunise animals in the lateral tarsal region just above the ankle. The immune response from this site is directed to the same draining lymph nodes, with a similar response, but there is minimal impact on mobility[11]. Following footpad injections animals must be given soft bedding, and may be provided with food and water in a gel placed on the floor of the cage to avoid the necessity for them to walk around. Social animals should be kept in groups but at a low stocking density so they do not have to climb over each other to reach food or water.

Intraperitoneal injections

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Intraperitoneal injections in rodents can be given into the caudal left or right quadrant of the ventral abdomen, to avoid the vital organs. The quadrants are demarcated by the midline and a line perpendicular to it passing through the umbilicus. In mice, both quadrants can be used, although in rats the animal's right quadrant may be preferred as the larger caecum can occupy the left quadrant. The animal should be held either by an assistant, or in one hand on its back, upright, so that it is comfortable and securely supported. The needle is inserted into the centre of one of the quadrants, angled towards the middle of the back. The needle is inserted a few millimetres, just enough to penetrate the body wall, and the injection given. There is no resistance to injection (Figure 7.7).

FIGURE 7.7 Intraperitoneal injection. Photo: James and Steve.



Transdermal administration

Some compounds can be introduced into the epidermis by transdermal administration. Particles can be coated onto gold particles, or liquids coupled to drug-delivery systems and fired through the skin using a gas gun. This is often used for vaccination with naked DNA in immunology studies. The advantages of this technique are that it is painless and good immunological responses can be obtained with small quantities of DNA. However, it does require specialised equipment and the specialised preparation and storage of the compound5. Depending on the species and site of administration, light anaesthesia or sedation may be required to allow site preparation and/or administration of the test material. Animals with dense fur such as rodents and rabbits should have the administration site clipped and shaved to remove as much hair as possible. The device or delivery system should be positioned so that it is in close contact with the skin, usually perpendicular to the skin surface. Following delivery the site can be monitored for dermal responses including erythema and oedema.

Oral administration

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Substances may be given orally by inclusion in the diet or drinking water. These have the disadvantages that either a whole group has to be dosed, or animals have to be singly housed. Also it is impossible to be sure that the animal has had the entire dose, and some animals may refuse to eat and drink if the feed or water is medicated. In mice particularly, adding drugs to the water can lead to dehydration, because avoidance of drinking by a mouse can lead to a rapid deterioration in the animal's condition. With *ad libitum* feeding, or if there is an increase in metabolic rate, the animal may overeat and thus ingest an overdose of the drug. If the watering system is automated it is impossible to give compounds in this way. To overcome these problems gastric intubation or gavage may be employed (Figure 7.8). Flexible catheters or stainless steel needles with rounded tips are used. Rigid needles may be straight or curved. The needle or catheter should first be measured against the animal, and marked at the length which reaches the xiphisternum. The animal is restrained with its neck extended, and the needle or catheter is passed in the midline at the top of the mouth and over the back of the tongue gently down the oesophagus, until the mark on the catheter is reached. Care must be taken not to damage the oesophagus, or to put the needle into the trachea. There should be minimal resistance as the catheter passes down the oesophagus, although there may be some resistance as the catheter passes through the sphincter into the stomach. The needle or catheter can usually be observed passing down the oesophagus on the left side of the neck. Damage to the catheter from chewing can be avoided by using an oral speculum, or by using a flexible nasogastric or pharyngostomy tube instead.

FIGURE 7.8 Gavage in a mouse. Photo: James and Steve.



For larger animals, such as dogs and cats, tablets may be administered orally in the conscious animal by placing one hand over the top of the head, placing the thumb at the commissure of the lips on one side and the fingers at the other, and tilting the head

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back. This will cause the mouth to open slightly. The tablet can be held between the thumb and forefinger of the other hand, and the middle finger used to open the mouth. The tablet can then be placed onto the tongue as far back as possible, to stimulate the swallowing reflex. This can be facilitated by holding the animal's mouth closed, tilting the head back and massaging the throat.

Legal considerations[12,13]

The UK Veterinary Medicines Regulations cover the administration of veterinary medicines to animals. These regulations are remade annually and therefore change slightly from year to year. The Veterinary Medicines Regulations 2011 came into force on 1 October of that year. The regulations prevent the supply of medicines for use in animals without an appropriate marketing authorisation, except in particular circumstances. Marketing authorisations do not generally cover laboratory species; therefore medicines used in these species are usually prescribed under an exemption. Supply of medicines for use in laboratory animals is primarily the responsibility of the designated veterinary surgeon, and researchers will usually have to obtain and use these medicines under the direction of a veterinary surgeon. A pharmacist may only supply licensed medicines for use in animals under the direction or prescription of a veterinary surgeon. Medical practitioners may not legally prescribe medicines for use in animals. It is an offence to supply out-of-date drugs.

The regulations categorise veterinary medicine according to the following.

- Prescription Only Medicine–Veterinarian (POM-V): these drugs may only be supplied or prescribed by a veterinary surgeon following a clinical assessment of the animal, or group of animals, under the veterinary surgeon's care.
- Prescription Only Medicine–Veterinarian, Pharmacist, Suitably Qualified Person (POM-VPS): these may be prescribed by any registered qualified person (RQP; a veterinarian, a pharmacist or suitably qualified person, or SQP). A clinical assessment of the animal is not required, but sufficient information about the animal must be known to prescribe appropriately.
- Non-Food Animal–Veterinarian, Pharmacist, Suitably Qualified Person (NFA-VPS): these may be supplied by an RQP as above provided the requirements for supply are met. These medicines do not require a prescription.
- Authorised Veterinary Medicine–General Sales List (AVM-GSL): there are no legal restrictions for the retail supply of veterinary medicines in this category, but a responsible approach to supply is expected.

Some drugs are also controlled under the UK Misuse of Drugs Regulations 2001[14]. These regulations place additional controls on the supply and use of drugs which are potentially addictive or dangerous. Drugs are classified according to five schedules, each with different requirements for supply, storage and record keeping. Veterinary medicines which are controlled drugs are in schedules 2, 3 or 4. Examples of schedule 2 controlled drugs include morphine and fentanyl. These drugs have to be kept in a locked cabinet, and acquisitions and individual uses recorded. Schedule 3 drugs include buprenorphine. These have to be kept in a locked cupboard but individual administrations do not have to be recorded. Schedule 4 drugs include ketamine and many benzodiazepines, and these must be kept securely and acquisitions recorded. There are strict requirements for the form controlled drugs registers have to take. See reference 14 for details.

Storage and record keeping

All drugs must be stored appropriately to ensure that they maintain their full activity. Some must be kept in a refrigerator at 2–5°C, some need to be kept away from light, some should not be kept in plastic bottles. The data sheet supplied with the drug will specify the requirements. It is good practice to keep a record of all drugs held and used, and it is a legal requirement to keep records of purchase and use of all controlled drugs listed in Schedules 1–3 of the Misuse of Drugs Regulations 200114. It is recommended that an annual check of the drug storage cupboard be carried out and any out-of-date drugs disposed, in accordance with any legal requirements.

Removal of Blood

The removal of blood from an animal has considerable potential to cause pain, suffering, distress or lasting harm. Possible adverse effects are listed below.

- Distress from handling and restraint: humane methods of handling and restraint must be used (see species chapters), and sedatives or anaesthetics may be needed. Training the animals to accept the handling required to take blood samples may also be beneficial.

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- Pain and discomfort: venipuncture requires considerable skill, and if carried out incompetently can lead to haemorrhage, bruising, thrombosis, embolism or phlebitis. In addition, poor sampling technique may cause the sample to clot or haemolyse, rendering results invalid.
- Hypovolaemia: the effects of removal of blood depend on the volume of blood removed, and the speed of withdrawal. The rapid removal of large quantities of blood may cause the animal to go into hypovolaemic shock, and may even cause death. The percentage blood loss required to cause hypovolaemic shock varies with the speed of withdrawal, whether or not fluid is replaced concurrently, and the physiological state of the animal at the time. Chronic slow haemorrhage is tolerated better than acute blood loss, and placid animals tolerate greater losses than nervous ones, reiterating the need for competent handling and training of the animals.

Stress responses in the animal result in the release of hormones and other substances to counteract the stress, which can cause anomalous experimental results. Expertise must be gained first by watching others, then by practising on cadavers or models, and then by carrying out the technique under direct supervision. The experimental technique should be refined such that the quantity of blood removed is minimised. This is particularly important in small mammals, such as mice, where the blood volume is small and sample volume is critical. It is essential to follow good practice guidelines such as the BVA/FRAME/RSPCA/UFAW (1993) working group report on the removal of blood from laboratory mammals and birds[15].

Quality of samples

To achieve meaningful results, samples must be of good quality, and be preserved in the best possible manner. Samples must be taken skillfully, and treated appropriately thereafter. Blood may be collected using syringes and hypodermic or butterfly needles, through indwelling cannulae, with double-ended needles and evacuated tubes (e.g. Vacutainers, Becton Dickinson), or in very small species by careful incision of a vein using a sterile lancet or scalpel blade and collection of the sample with a capillary tube. If needles are used, the needle should be as large as is practicable for the species. This allows blood to flow faster, reducing the likelihood of clotting, and also causes less damage to the red cells, reducing the possibility of haemolysis.

If no anticoagulant is used, the blood will clot, and serum can be removed after centrifugation. Alternatively blood may be collected into an anticoagulant. Different anticlotting agents are available, including the following.

- Lithium heparin: this is the anticoagulant of choice for most biochemical assays. The yield of plasma from heparinised blood is greater than the yield of serum from clotted blood, which may make heparin a good choice for collecting blood for harvesting antibodies. Sodium heparin is sometimes used if preservation of the white cells is required.
- Potassium ethylene diamine tetra-acetic acid (EDTA): this is used for haematological analyses as it preserves the cells.
- Oxalate/fluoride: this is used for blood glucose determination.

Several other anticoagulants are available, for example for collecting blood for transfusions or analysis of clotting factors. After collection into anticoagulant, the blood should be mixed thoroughly by *rolling*, not by shaking, as this can damage the cells and lead to haemolysis.

It is preferable for samples to be submitted fresh for analysis. If this is impossible, samples may need to be refrigerated, or deep-frozen after separation of cells and plasma or serum. It is advisable to determine the exact requirements of the laboratory protocol prior to sample collection.

Technique for venipuncture

Site and location of the vein

It is important to be certain of the location of the vein, either by visualising it or palpating its course, and to have it immobilised, before piercing the skin. If unsure of the position of the vein, venipuncture should not be attempted. Sometimes it is necessary to raise the vein, by occluding the venous drainage proximal to the site of venipuncture. This must be performed correctly, or withdrawal of blood will be difficult. The use of a quick-release tourniquet (Figure 7.5a) facilitates blood sampling in some species, and vasodilating agents may be used in others. Warming the animal in a thermostatically controlled hot box for 30 min at 37°C or dipping the tail in warm water can help dilate the tail veins in small rodents, facilitating venipuncture.

Preparation of the site

Blood should be collected using aseptic technique. The area should be clipped to remove hair if necessary, and then cleaned using a

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suitable antibacterial cleanser such as chlorhexidine. Iodine-based preparations may also be used but are less persistent. The use of warm water with or without disinfectant will help dilate superficial veins as well as cleansing the skin. After cleansing, the skin can be swabbed with 70% ethanol or disinfectant, which helps make the vein stand out as well as disinfecting the skin. It may be advantageous to apply local anaesthetic cream (e.g. EMLA, AstraZeneca) to the site 30–60 min before venipuncture to prevent any discomfort.

Taking the sample

For needle venipuncture, the needle should be held bevel uppermost and directed through the skin following the course of the vein. Once in the lumen of the vein, as determined by the presence of blood in the needle hub, the needle should be advanced parallel to the skin up to the hub, so that the body of the needle is in the lumen of the vein. The needle may be bent at an angle, if required, to facilitate location in the vein; for example, in the jugular vein of the ferret (see Figure 7.6).

A similar method can be used for butterfly needles. Over-the-needle cannulae can be used in almost all species, except possibly mice. Choose the largest, longest cannula that will pass into the vein. Clip the fur from a wide area over the vein, and clean the skin as above. Raise and immobilise the vein (an assistant may be required for this), remove the bung from the stylet which runs through the cannula and insert it through the skin until the tip is in the lumen of the vessel and blood is seen in the hub of the stylet. For thick-skinned animals it may be worth making a tiny skin incision first, using the bevelled edge of a needle or a no. 11 scalpel blade, to facilitate passing the cannula through the skin. The cannula can then be advanced into the vein while retaining the stylet: this prevents the stylet from lacerating the vein as it advances up the vessel, and also the stylet blocks the lumen of the cannula so blood may only be withdrawn once the stylet is removed. Flexible cannulae are less traumatic to the tissues than needles, and are therefore less painful to insert and more suitable for long-term cannulation.

Veins will collapse around the needle if attempts are made to withdraw the blood too quickly, so patience is required, particularly with small mammals. For these species, blood can be allowed to drip from a needle or flexible cannula placed in the vein. A syringe may be attached and gentle suction applied for larger animals. Evacuated tubes can be used if the vein diameter allows it. These are quick and easy to use, but can increase the likelihood of haemolysis as blood cells can become damaged by the rapid passage into the low-pressure container. After blood collection, a swab should be applied to the site and the needle removed, while pressure is applied to the vein for 30–60 s to prevent haemorrhage. The site should then be checked to ensure bleeding has stopped and further pressure applied if necessary.

Potential sequelae

Haemorrhage may occur from the punctured vein. If this occurs, pressure should be applied until the haemorrhage ceases. Dressings are available which will accelerate haemostasis (e.g. Kaltostat, Convatec). Bruising may occur if the vein bleeds under the skin. Pressure should be applied as above, and the site rechecked after 30 min. If the bruised area continues to spread, advice should be sought from a veterinary surgeon. The occurrence of thrombosis or phlebitis following venipuncture indicates poor technique, and the method should be reviewed and advice sought from a veterinary surgeon.

Sample volume

The volume of the sample taken is determined by the requirements of the experiment, and by the safe limit which can be withdrawn without causing distress to the animal. In general, as small a volume as possible should be taken. As a general rule, for mild severity not more than 10% of the blood volume should be removed at one time, and less than 15% of the blood volume should be removed in any 30 day period. Animals have approximately 70 ml of blood per kilogram of body weight, but this varies with the species (see Table 7.3), and withdrawal of a smaller volume may have detrimental effects if the animal is compromised. Larger volumes may be withdrawn with few effects if there is concurrent volume replacement. Maximum limits of volume and frequency of sampling will normally be stipulated in the experimental protocol.

Table 7.3 (a) Practical blood-sampling volumes for laboratory species. (b) Practical blood-sampling volumes for larger domestic species.

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(a)

Species	Reference weight	Blood volume (ml/kg)	Total blood volume, normal adult (ml)	Safe volume of single bleed (ml)*	Bleed-out volume (ml)
Mouse	18–40 g	58.5	Male 1.5–2.4 Female 1.0–2.4	0.1–0.2	Male 0.8–1.4 Female 0.6–1.4
Rat	250–500 g	54–70	Male 29–33 Female 16–19	Male 2.9–3.3 Female 1.6–1.9	Male 13–15 Female 7.5–9
Hamster	85–150 g	78	Male 6.3–9.7 Female 7.1–11.2	Male 0.6–0.9 Female 0.7–1.1	Male 2.9–4.5 Female 3.3–5.2
Gerbil	55–100 g	66–78	Male 4.5–7 Female 3.8–6	Male 0.4–0.7 Female 0.4–0.6	Male 2.2–3.5 Female 1.9–2.9
Guinea pig	700 g–1.2 kg	69–75	Male 59–84 Female 48–63	Male 6–8 Female 5–6	Male 29–42 Female 24–31
Rabbit	1–6 kg	57–65	57–390	6–40	3–200
Ferret	600 g–2 kg	70	42–140	4–14	21–70
Marmoset	350 g	60–70	21–24.5	2.1–2.4	
Rhesus	Male 6–11 kg Female 4–9 kg	55–80	Male 420–770 Female 280–630	Male 42–77 Female 28–63	
Cynomolgus	Male 4–8 kg Female 2–6 kg	50–96	Male 280–560 Female 140–420	Male 28–56 Female 14–42	

*A single bleed of 10% of the blood volume averages 7 ml/kg.

(b)

Species	Blood volume (ml/kg)	Total blood volume, normal adult (ml)	Safe volume, single bleed (ml)*
Dog	70–110†	900–1170‡	90–110
Cat	47–65	140–200	14–20
Pig, Large white	56–69	13 200–15 000	1320–1500
Pig, Yucatan	56–69	4200–4800	420–480
Sheep	58–64	4060–4480	400–450
Goat	57–90	3990–6300	400–630
Cattle	60	27 000–36 000§	2700–3600
Horse	75	33 750–45 000§	3375–4500

*Single bleed of 10% of blood volume.
†Much breed variation.
‡Beagle.
§Assumes adult weight 450–600 kg.

Methods of venipuncture in different species

Rodents

In rodents with tails, the usual site for venipuncture is the tail vein. The lateral tail veins are usually used as they are larger than the dorsal and ventral veins. Warming the tail first increases the blood flow to the site and makes sample collection easier. Blood samples may be obtained by incising the skin over the vein using a sterile lancet and collecting the blood into a plain or anticoagulant-coated capillary tube, or allowing it to drip into a container. Scalpel blades are not recommended as they can easily slip, causing damage to the tail. Larger samples can be obtained from rats by placing a 23–26 G flexible cannula or butterfly needle into the tail vein and allowing blood to drip into a collection pot or applying gentle suction with a syringe. If a butterfly needle is

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used, the plastic tube should be shortened to prevent clotting within it.

For mice and tail-less animals, the saphenous vein can be used[16] (see also http://film.oslovet.veths.no/). The vein is punctured using 23 or 25 G needle, a drop of blood forms at the puncture site and can be collected into a microcapillary tube. The scab that forms at the site can be gently rubbed off to enable serial samples to be collected from the same puncture site. In larger animals this vein can be cannulated[17].

Amputation of the tail tip to obtain a sample of mixed arterial and venous blood can be carried out on rats, mice and gerbils, but must be done under general anaesthetic21. In mice, this does not appear to involve the removal of any vertebrae, which it does in rats. This should only be performed on a single occasion.

Large samples can be obtained from rats and gerbils via jugular venipuncture, usually under general anaesthesia. The vein is raised on one side of the neck by applying pressure at the thoracic inlet, and a needle placed through the skin and into the vein pointing towards the head.

Blood can also be obtained by puncturing the superficial temporal vein on the face using a sterile lancet[18]. The facial veins run up from the neck and divide at the angle of the jaw into the superficial temporal vein which runs to the eye, and the facial vein which runs along the jaw line. Hold the mouse by a large pinch of the scruff of its neck on the left side: the eyes should be bulging slightly. The vein can be punctured as it runs along the lower jaw, directly below the lateral canthus of the eye. In white mice there is a grey dot marking the sebaceous gland on the jaw, and the puncture site is found by moving one eye length back and one eye width up from this gland. In black mice there is a black dot marking the gland. The vein should be punctured boldly with the lancet, and the blood should be allowed to fall into the collecting vessel to avoid haemolysis. Blood flows freely from this site if carried out correctly and care must be taken not to take an excess of blood. This technique requires training and practise before use and anaesthesia is advised (see reference 19).

For some animals with no tails, such as guinea pigs, tiny samples can be taken from the ear veins.

For terminal sampling it is acceptable to perform cardiac puncture, which must be done under terminal general anaesthesia. There are many potentially harmful sequelae to this procedure, such as cardiac tamponade. The heart may be reached by placing the animal on its right side and piercing the left ventricle through the chest wall at the sixth intercostal space, one third of the way up, to obtain arterial blood, or by piercing the right ventricle with the animal on its left side for venous blood. Alternatively, the animal may be placed on its back, and the heart reached by passing the needle under the sternum and through the diaphragm.

Rabbits

Blood can be collected relatively easily from the marginal ear vein using an over-the-needle cannula or butterfly needle. The skin over the vein should be clipped and cleaned using a suitable disinfectant, then 30–60 min before venipuncture local anaesthetic cream should be applied. A peripheral vasodilator may also be applied, 5–10 min before blood collection. Once the vein is engorged, the cannula is inserted and blood can be collected by allowing it to drip into a pot. After collection, the vasodilator is wiped off and pressure applied until the bleeding ceases. Bleeding from the central ear artery is possible, but can result in the formation of large haematomata, which can cause damage to the ear or even necrosis.

Ferrets

For tiny quantities of blood, a toenail can be clipped and a drop of blood collected into a capillary tube. For moderate-sized samples the saphenous or ventral tail vein can be used, while larger quantities can be collected from the jugular vein. The fur on the neck needs to be well clipped, and the vein raised by placing a thumb over the jugular groove in the thoracic inlet. The needle can be inserted pointing up the vein towards the head, or down towards the thoracic inlet. The skin is very thick, and some pressure may be needed to penetrate the skin. Collection can be facilitated by bending the needle to an angle of 30° prior to penetrating the skin, or by inserting the needle through the skin parallel to the vein and then into the vein. Pressure on the vein in the thoracic inlet is maintained until the blood has been collected (see Figure 7.6).

Primates

The best method of blood withdrawal in primates is to use the femoral vein, in the groin. This vein is not readily visible and its position has to be determined by palpation. The needle is inserted in the femoral triangle, slightly medial to the femoral pulse, and directed in a craniomedial direction. The needle should be advanced slowly until the vessel is reached, as determined by blood in the hub of the needle. For larger Old World monkeys the cephalic vein on the top of the foreleg below the elbow can be used, as for cats and dogs. The jugular vein can be used as an alternative route. A microcapillary tube can be used to collect small samples

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from the heel of primates without anaesthetic after they have been trained to accept minimal restraint. Marmosets may also be bled from the coccygeal vein.

Dogs and cats

Small and medium samples can be collected from the cephalic vein one the foreleg. The skin over the anterior aspect of the foreleg is prepared by clipping and cleaning, then a handler restrains the animal by placing one arm over the animal's body, holding the leg forward by placing two fingers behind the animal's elbow, raising the vein with the thumb over the top of the leg and restraining the animal's hindquarters against the body with the elbow. The other hand is placed under the chin to raise the animal's the head. They can also be bled from the jugular vein. A handler places their right arm over the body of the animal to hold the forelegs. The elbow is used to hold the body of the animal to the body of the handler (Figure 7.9). The left hand is placed under the chin to raise the head. The person collecting the blood raises the vein by placing a thumb in the jugular groove at the thoracic inlet.

FIGURE 7.9 Holding a cat for jugular venipuncture.

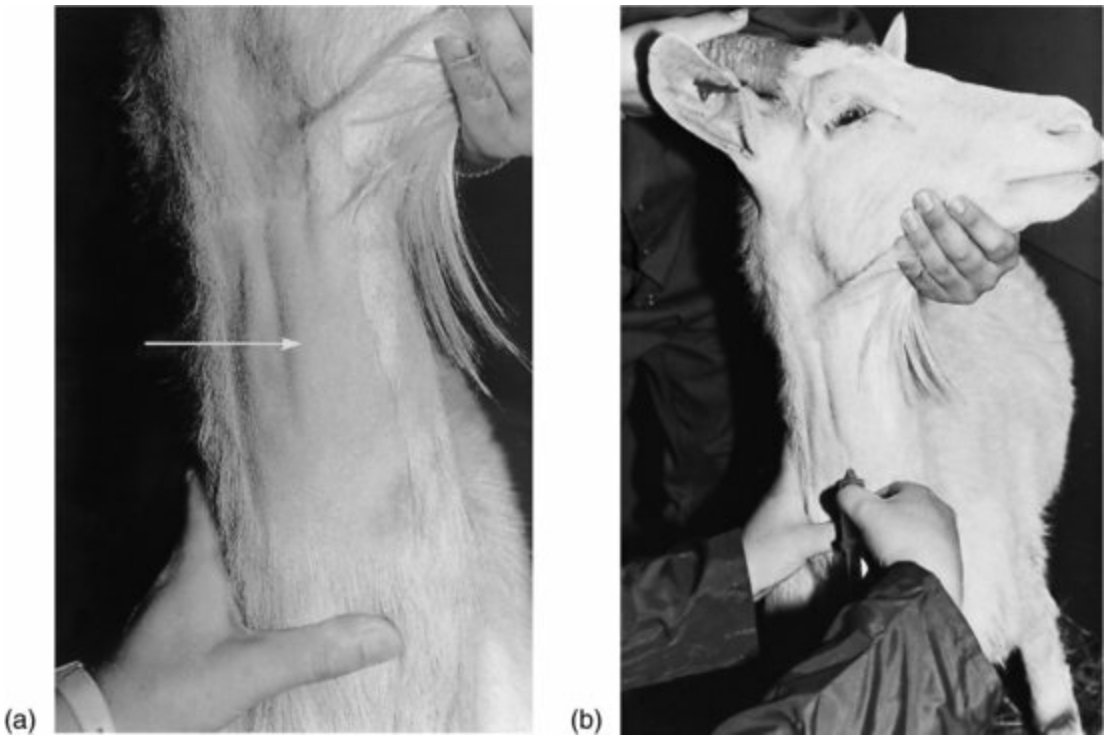


Ruminants and horses

For sheep, goats, cattle and horses the jugular vein is used. The vein can be visualised by clipping hair or wool from over the jugular groove. The vein is raised by applying firm pressure to the base of the jugular groove. The needle is advanced through the skin up towards the head (Figure 7.10). Cattle can also be bled from the ventral coccygeal vein.

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FIGURE 7.10 (a) Visualising the jugular vein in a goat. (b) Jugular venipuncture in a goat.



Pigs

These are probably the most difficult animals to bleed. For small volumes in large pigs an ear vein can be used. For larger volumes, the anterior vena cava is used. The animal is restrained in a snout snare with the head held slightly upwards. A long needle is inserted in the thoracic inlet, and angled slightly upwards and medially to enter the anterior vena cava. Smaller pigs can be restrained on their backs for this. Snout snare should be made of smooth material and only applied for a few minutes as they can be uncomfortable[20].

Birds

The brachial (alar or wing) vein is usually used for blood sampling in birds. It can be visualised as it crosses the elbow by plucking the feathers over the medial surface of the wing. Samples should be removed slowly to prevent the vein collapsing. Pressure is generally not applied after venipuncture in birds as this can promote haematoma formation. The right jugular vein can also be used. It is found between the feathers on the dorsolateral surface of the neck. It can be raised by applying pressure at the base of the neck. Haematomata rarely form after jugular venipuncture.

Placement and Maintenance of Indwelling Cannulae

Indwelling vascular cannulae can be placed for prolonged periods if maintained properly. Butterfly needles may be used for short-term cannulation, but flexible plastic cannulae are preferred for longer-term experiments, since butterfly needles may lacerate the vein if there is any movement. Cannulae need to be of a suitable inert material, which will not cause any irritation, and be strong enough to withstand kinking and occlusion due to movement or tightening of ligatures. Polypropylene or silicone cannulae are available.

Cannulae may be implanted surgically, or passed into a superficial vein by puncture of the skin, as for needle venipuncture. Percutaneous insertions are associated with fewer complications. Placement of all cannulae should be performed under aseptic conditions. Cannulae can be placed in a major vessel, or into a minor one with the tip passing into a major vessel. The cannula may be tunnelled through the subcutis to exit the skin at a distant site (e.g. over the back) to reduce interference from the animal

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and the risk of ascending infection, taking care that the cannula does not kink or pull out when the animal moves. Once the cannula is in place, it should be capped using an injection bung or a three-way tap, and sutured or bandaged to the skin. To prevent clotting in the cannula, it should be flushed with heparinised saline or another anticoagulant after placement, and between sampling or administration of compounds. Three-way taps or multiple sampling ports facilitate administration as the anticoagulant delivery and sampling/administrating systems may be attached at the same time. Exit sites are potential sources of infection and irritation, and must be inspected at least once daily. The skin around the cannula should be cleaned with suitable antiseptic every 24 h and checked for signs of inflammation.

Maintenance of cannulae for repeated administration is easier than for repeated withdrawal, as blood clots on the end of the cannula tend to allow administration but act as valves preventing withdrawal. Wipe the injection port with spirit prior to injection, and keep the ports of three-way taps capped. Substances to be given through the cannula must be sterile, and fluid-giving sets must be changed every 48 h.

Withdrawal of blood

When withdrawing blood via a cannula, the first sample taken each time should be discarded, as it will contain anticoagulant and be diluted. After sampling, the cannula should be flushed with a calculated amount of anticoagulant: enough to fill the cannula, but not to enter the bloodstream.

Long-term cannulation

Long-term cannulae are usually implanted surgically, which must be done under aseptic conditions and requires considerable skill. Cannulae will last longer if placed with the flow of blood, and may be maintained for prolonged periods provided the flow of fluid is not interrupted and the cannula is flushed frequently with a suitable anticoagulant. In young animals room must be left so the cannula is not pulled out as the animal grows, and in all animals movement can be a problem. Cannulae can be kept patent for more than 3 or 4 weeks in small animals, and longer in large animals. The period can be increased if the animal is tethered, but this can result in distress to the animal and is best avoided.

Removal of cannulae

After removal, haemostasis can be achieved by applying pressure to the vessel for several minutes, or by ligating the vessel with a suitable suture material.

Potential sequelae

If cannulae are maintained for longer than 24–48 h blockages due to thrombi can become a serious problem. Thrombi may embolise and travel to distant sites, and will be filtered out in a capillary bed depending on the site of the cannula. Emboli originating from jugular catheters will travel through the right heart and be trapped in the capillary beds of the lungs, whereas emboli from arterial cannulae lodge in end organs such as the brain, kidney or muscles. Cannulae exiting through the skin can act as entry sites for infection if not properly maintained.

Arterial Sampling and Arterial Loops

Arteries may be sampled when very large samples are required, or for arterial blood gas analysis, for example. Arterial sampling allows very large samples to be taken rapidly. Often, the carotid artery is used, but the central ear artery can be used in rabbits, or the femoral artery in many other species.

Needle puncture

Good restraint is essential when taking samples with a needle as accidental movement can result in laceration of the artery and profuse haemorrhage. Once the needle is in the artery the high blood pressure will normally force the plunger in the syringe back. Firm pressure should be applied to the site for 2–5 min afterwards to prevent haematoma formation.

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Arterial cannulae

Arterial cannulae are usually placed surgically under aseptic conditions. The artery should be exposed and clamped with atraumatic vascular clamps, then the cannula inserted into the vessel and tied in place before the clamps are released. Otherwise there will be haemorrhage as soon as the artery is punctured. Cannulae should be placed in the direction of the flow of blood. There is a greater potential for thrombi to cause problems in arterial cannulae than venous ones.

Arterial loops

This method involves bringing an artery into a subcutaneous position for easy sampling. This has to be done surgically, and is technically difficult.

Miscellaneous Procedures

Creation and maintenance of fistulae

A fistula is an artificial passage created between two hollow organs, or between a hollow organ and the outside. For example, ruminal fistulae may be created to study the physiology of the rumen in sheep, goats and cattle. Fistulae are created surgically under general anaesthetic. An incision is made in the skin overlying the organ, and the organ is brought up to the surface. The wall of the organ is sutured to the skin edge to make a seal, before the organ is punctured and the mucus membrane from the inside of the organ sutured to the skin to create a mucocutaneous junction. A bung may be inserted into the fistula to close it and to prevent closure of the fistula. Aftercare involves checking and cleaning daily to prevent early closure, or damage to the skin around the fistula from exudation of serum or organ contents. At the end of the experiment, the fistula may be closed surgically, or allowed to heal over by second-intention healing.

Administration techniques

Administration via osmotic minipumps and other slow-release devices

Substances needing prolonged administration may be administered via a slow-release device. Osmotic minipumps can be implanted subcutaneously or intraperitoneally, and can release compounds over periods of up to 4 weeks. They can be connected to implanted cannulae to permit intravenous or intracerebral administration.

Nebulisation/administration into the respiratory tract

The compound may be dripped into the external nares, or the animal placed in an inhalation chamber and the compound given in a spray or as a vapour. Some liquids may be nebulised into tiny particles for administration via inhalation. The compound may be administered to the entire body in a chamber, or just to the nose via specialised inhalation devices.

Instillation into eyes

Compounds for topical ocular administration must be non-irritant, or there may be discomfort, lacrimation and corneal damage, with scarring in the long term.

Intracerebral injection

This technique is used to deliver pharmacological agents to the central nervous system, either where the blood–brain barrier must be crossed or to avoid direct systemic effects. This should always be done under aseptic conditions as the central nervous system is highly susceptible to infection. In neonatal rodents and chicks this can be done with a small-bore, low-volume (less than 0.5 ml) plastic syringe with a 27 G needle, and it is recommended that this is done under general anaesthesia. Neonatal animals require very careful, gentle handling and it is essential to gain experience in handling them prior to the procedure. Hold the animal gently

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but firmly, by resting it on an absorbent pad on a firm surface with the dorsal surface of the animal held uppermost between thumb and forefinger. Insert the tip of the needle into the brain, usually from the front, at an angle of 45°, halfway between the eye and the ear, in the area of the anterior cranial fontanelle, and gently expel the dose. Return the animal to its mother, check for adverse effects after 1 h (to avoid too much immediate disturbance) and monitor closely at least daily post-injection for adverse effects. Occasionally, the mother rejects the young when they are returned to her. If there is blood leaking from the injection site she may eat them. No more than 10 and 30 µl of solution can be injected into neonatal mice.

In adult rodents a stereotaxic frame is needed to ensure that there is no lateral movement of the needle, which would cause trauma. Anaesthesia and aseptic technique are essential. Injection volumes should not exceed 30 µl, and should be given slowly enough to avoid increasing cerebrospinal fluid pressure. Penetration of the ventricle is readily assessed by the sudden reduction of back pressure in the injection line. Chronic placement of cannulae allows injections, or continuous intraventricular infusion, without need for repeated anaesthesia.

Alternative methods of obtaining the results required should always be considered before using this route. The procedure is not easy to perform, and incorrect placement of the needle can kill the animal. Any animal showing unexpected neural deficits, for example, ataxia or paralysis, within the first 24–36 h should be killed as this is most likely to be due to faulty technique.

Intra-articular injection

Injections into joints *must* be carried out under aseptic conditions to avoid causing septic arthritis. Compounds may be administered by this route for experimental reasons, or samples of joint fluid may be taken for analysis. It is essential to be certain of the anatomy of the joint and its synovial capsule before penetrating the skin, so as to be certain of entering the correct part of the joint.

Per rectum

Enemas may be given to empty the rectum before bowel surgery or radiography, or fluids and some drugs may be given into the rectum or distal colon. Substances may be given via a flexible rubber or polythene tube, or a rigid sigmoidoscope. Whichever method is used, it is essential that the instrument is well lubricated and inserted very gently.

Sampling

DNA

Samples of tissue for DNA analysis can be performed following DNA amplification by PCR on blood cells or cells from a cheek swab. A technique has been described for sampling of cheek cells from neonatal mice[21]. This method is minimally invasive and is to be preferred to invasive techniques such as ear punch or tail biopsy. If these methods are to be used, anaesthesia should be given.

Urine

Urine may be collected as it is voided naturally in all species (rodents often urinate or defaecate when picked up), or by using various techniques. A metabolism cage, in which the animal's waste products can be collected, can be used in all species, and in rodents this is the only reliable method for obtaining a sample. For larger species urine may be expressed manually, collected by using a urethral catheter if the urethra is wide enough (e.g. rabbits and larger species) or withdrawn by cystocentesis under aseptic conditions. With the latter method, the skin over the lower abdomen is clipped and cleaned, the full bladder is immobilised with one hand and a sterile needle of a suitable gauge (see Table 7.2a) inserted through the body wall into the bladder.

Urinary catheters may be maintained in situ for prolonged periods, either after surgical implantation (in which the catheter may be channelled to exit the skin at a distant site as for venous cannulae), or passing into the urethra. Urinary catheters predispose to cystitis and urethritis, so particular care must be taken when maintaining them for prolonged periods, and they should not be kept in place for any longer than necessary. Clean the prepuce or vulva prior to insertion of the catheter using an antibacterial cleanser. Use a soft, flexible, sterile catheter and lubricate it before insertion. It can be sutured in place to prevent movement. The catheter should not be introduced too far into the bladder to avoid trauma to the bladder wall. Urine can be collected into a bag. To avoid urine scald, the area around the catheter must be cleaned and the protected with petroleum jelly or a similar barrier. To prevent infection from tracking up the catheter into the urinary tract, make sure urine always drains out of the

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bladder and clean the vulva or prepuce around the catheter daily with antibacterial cleanser, and the catheter can be flushed with sterile saline or dilute chlorhexidine if necessary.

Faeces

Faeces may be collected after being voided naturally, by using an enema or by using a rectal swab. A metabolism cage can be used, and in rodents again this is the most reliable method. Enemas may be given to collect large quantities of faeces, or swabs may be used for small samples, for example for bacteriology. It is essential that anything passed into the rectum is inserted gently. In rodents and rabbits, which are coprophagic, hard faeces can be collected as above, but to collect soft faeces, which are normally eaten straight from the anus, restraining devices must be used.

Milk

The mammary glands of ruminants and horses are served by teats, whereas those of the smaller animals have nipples. Teats are larger, with one or two orifices from which milk can be expressed, and the udders of these species are large enough to permit the taking of large samples. At peak lactation, up to 30 l of milk may be withdrawn twice daily from a Friesian-Holstein cow. Nipples have several orifices from which milk flows, and small samples can be collected from these. Milk can be expressed by grasping the nipple or teat at its base and gently pulling towards the tip. In newly parturient females, injections of oxytocin may increase the release of milk. Several methods have been described for collecting milk from mice and rats[22,23]. These usually involve separating the pups from the mother and keeping them warm for 2–3 h prior to milking. For mice, the dam is given 2 IU of oxytocin by intraperitoneal or subcutaneous injection. Milk collection should be started within 1–2 min. Milk can be withdrawn by manual suction via a modified pipette tip or automatically using specialised equipment. Milk yield is highest in the first 1–2 weeks of lactation and declines in the third week.

Antibody production

Antibodies are used in a huge variety of studies, ranging from medical research to archaeological and forensic enquiries, and are also used therapeutically. Antibodies have traditionally been created by injecting antigens into animals and harvesting the antibodies. However, these methods have limitations: the volume of antibody produced may be small, and the use of mouse immunoglobulins may cause immune reactions if used therapeutically. Therefore increasingly sophisticated methods of producing of antibodies *in vitro* are being developed. Generating monoclonal antibodies *in vitro* using murine hybridoma cells allows large quantities of specific antibody to be produced facilitating diagnosis and therapeutic use. However, this still requires animals to supply the initial immune cells. Technical difficulties in producing human hybridomas led to the development of molecular approaches that allow the production of human antibodies without the need for B-cell transformation. These technologies involve the cloning of immunoglobulin gene segments to create immune libraries from which antibodies with desired specificities can be selected[24]. These technologies will play an increasing role in the production of reagents for diagnostic and therapeutic use.

Antibodies may be polyclonal (with mixed specificity), or monoclonal (with a single specificity).

Polyclonal antibody production

Polyclonal antibodies are a mixture of immunoglobulins that recognise different epitopes on a specific antigen. The quality of antibody produced depends on the animal species, route of immunisation, type and quality of the adjuvant and method of blood collection. However, other factors can also affect the antibody and/or the welfare of the animals, including the health and genetic status, the competence of staff and the housing of the animals[25]. Polyclonal antibody production can be achieved in a relatively short time, from 4 to 8 weeks.

The choice of species is important. When there is no need for a specific species, animals that are relatively easy to bleed should be used (e.g. rabbits and mice), rather than those which are difficult to bleed (e.g. guinea pigs and hamsters). The purpose of the experiment should be considered also, as each species, strain, stock or breed of animal might differ in its immune response.

Ideally, the animal should be of a different species from the donor of the antigen to ensure a maximal immune response. Rabbits are good for making antibodies to other mammals. Rats are good for creating IgE antibodies, and farm animal species are useful if large volumes of serum are required. Chickens can be used as an alternative to mammals, and the production of avian antibodies (IgY) in chickens is considered to be a refinement, since the collection of blood is not required as antibodies can be extracted from egg yolk25.

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The route of injection should distribute the antigen to the largest number of lymph nodes possible to maximise the immune response, while minimising the effects on the animals. Intradermal inoculation is the most effective route for soluble antigens without adjuvant, but tends to produce the most local reaction. The adjuvant remains undispersed, and is exposed to the immune system for a prolonged period, leading to the formation of large, usually non-painful granulomas. Intramuscular administration can be painful, particularly if adjuvants are used. Subcutaneous injections are easier and produce fewer granulomas, although the adjuvant may track ventrally, producing fistulous tracts and firm linear lesions distant from the injection site. Particulate antigens and those with adjuvant are best given by the subcutaneous route to minimise adverse effects although intramuscular or intradermal routes may sometimes be used. For antigen without adjuvant, intravenous, subcutaneous, intramuscular, intradermal or intraperitoneal routes may be used, although there is a risk of anaphylaxis if booster injections are given via the intravenous or intraperitoneal routes.

The antigen or antigen/adjuvant mixture should be prepared aseptically to minimise the risk of contamination, and the injection given using sterile technique. Any adjuvant-induced granulomas are composed mainly of macrophages and lymphocytes, and are non-painful. The introduction of bacteria causes an infiltration of polymorphonuclear leucocytes, which tends to be painful. This predisposes to self-mutilation by the animal.

Typically, animals are given a priming injection, test bled to determine antibody titres and booster injections then given at intervals. In general, a booster is given after the antibody titre has plateaued or begun to decline. If the first immunisation is performed without adjuvant, the antibody titre will usually peak 2–3 weeks after immunisation. When adjuvant is used, a booster may not be needed until at least 4 weeks after the first immunisation. For booster immunisations an adjuvant is not always necessary25. Animals can be rested for long intervals between boosting. Even when serum antibody titres have dropped to relatively low levels, a booster injection will usually re-establish a high serum antibody titre. Regular blood collection after immunisation appears to help maintain a high serum antibody level. However, animals must not be kept in antibody production programmes indefinitely. See Table 7.4 for recommended volumes for injection of adjuvants.

Table 7.4 Recommended injection volumes for immunisations[25,26].

Route	Species	Volume of single dose (ml)	Volume of maximum total dose (ml)*
Intradermal	Rabbit	0.05–0.1	0.4
Intramuscular	Rabbit	0.1–0.25	0.5
	Mouse, hamster	0.05	
	Guinea pig	0.25	1
Subcutaneous	Mouse, hamster	0.1	0.2
	Rabbit	0.1–0.25	1.0
	Rat, guinea pig	0.2	0.4
	Sheep and goats	0.25	1
	Chicken	0.5	

*In rabbits, guinea pigs, sheep and goats a maximum of four sites should be used, and in rats and mice a maximum of two sites should be used. For intramuscular injections in mice or hamsters, a single hind limb should be injected.

Adjuvants

Some antigens must be given with an adjuvant, to enhance the immune response. Adjuvants are usually not necessary when whole bacteria, whole cells or other particulate antigens are used, but may be necessary for soluble antigens. Adjuvants might also be necessary when only a very limited amount of antigen is available, when native antigens are used or when a specific type of response is required. The ideal adjuvant should stimulate high and sustainable antibody titres, work in a variety of species with a broad range of antigens, be easily prepared in an injection mixture and easily injectable, be effective in a small number of injections, have low toxicity for the animal and not be harmful to the investigator. No single adjuvant is ideal. Modern synthetic adjuvants produce good responses with minimal adverse effects. These are to be preferred to the use of complete Freund's adjuvant, which uses mycobacterial antigens and mineral oil to stimulate the immune response, and is associated with significant local reactions. If Freund's adjuvant is to be used, the complete adjuvant should only be given once to a single animal, with boosts to be given with incomplete Freund's adjuvant, in which the mycobacterial component is absent.

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Sampling

Superficial blood vessels are usually adequate for blood collection during monitoring of antibody titre in all species. No more than 15% of total blood volume (TBV) should be taken over any 4 week period and usually no more than 10% TBV should be removed in a single collection. The TBV of laboratory animal species averages 70 ml/kg (see Table 7.3). Local or general anaesthesia should be used as appropriate for the species.

Harvesting

When the antibody titre has reached a plateau, mammals should be bled out under terminal general anaesthesia. The serum can then be separated and stored for use. Where serial harvesting is appropriate, the volume of blood collected should be limited as set out above, unless special measures such as volume replacement and haematological monitoring are authorised. If chickens are used, eggs should be collected during a laying season.

Monoclonal antibody production

Monoclonal antibodies recognise only one epitope on an antigen. They are produced by first immunising mice against the antigen of interest, then harvesting the spleen to isolate cells producing the required antibody. A single B-cell can then be fused with a myeloma cell line to make a hybridoma, which is an immortal cell line, and the antibody is then secreted by this clone of cells. Once a single, stable clone is isolated, the antibodies can be made either *in vivo* with ascites, or by *in vitro* cell culture. *In vitro* methods should be used wherever possible, and this is usually the case26. If the cells do not grow in culture, antibody can be created through the production of ascites fluid. This is done by injecting the hybridoma cell line into the primed peritoneal cavity of mice and harvesting the resulting ascites fluid that contains antibodies secreted by the hybridoma. The production of ascites can be painful and this method requires particular justification. Monoclonal antibody production can take time, up to 3–6 months.

Equipment for Blood Collection and Administration of Substances

Over-the-needle cannulae

Flexible over-the-needle cannulae can be obtained in a variety of sizes to suit the species and the intended site of administration or withdrawal. They are easily inserted into the superficial veins of most species, and once *in situ* they do not lacerate the vein if the animal moves. For recommended sizes see Table 7.2b.

Evacuated blood-collection tubes

These are tubes with a rubber seal on one end which contain a partial vacuum (e.g. Vacutainers). Double-ended needles and a special holder are required to use evacuated tubes. The needle is screwed into the holder, with one end of the needle just touching the rubber seal of the tube without penetrating it. The other end of the needle is placed into the vein, and the tube advanced until the needle penetrates the seal. The vacuum in the tube sucks blood into the tube. If the tube is advanced too soon, then air will be sucked into the tube and the vacuum will be lost. Once the tube is full, no more blood is sucked in. The tube should be taken out of the holder as the needle is withdrawn from the animal. Evacuated tubes are quick and easy to use, but are only useful if the vein is large enough to allow rapid flow of blood. Occasionally, the rapid passage into the evacuated tube will damage the red blood cells and result in haemolysis. Evacuated tubes are available with or without anticoagulant: if anticoagulant is required, mix it with the blood by rolling the tube, not shaking it, as this can damage the cells.

Butterfly needles

These are needles with a long flexible tube attached, with a fitting on the end for syringes. They are useful because a syringe can be attached at some distance from the animal, and the needle can be taped in place in a vein using the wings. They can be used for administration of substances and withdrawal of blood.

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Tourniquets

When performing intravenous injections or withdrawing blood from some superficial veins, the vein must be raised before venipuncture. This can be done by an assistant, placing their thumb over the vein proximal to the site of venipuncture, but this can be awkward, and it is often easier to use a tourniquet. These occlude the venous drainage, raising the vein, and usually have quick-release mechanisms so they can be removed rapidly (see Figure 7.5a).

Vasodilating agents

For some smaller species, for example, mice, rabbits and rats, collecting blood can be facilitated by applying vasodilators to a superficial vein to engorge it and increase the blood flow. Warm water can be used to warm the tail, or the animal placed in a warm box at 37°C for 30 min. Alternatively chemical agents are available, which cause vasodilation within 5–10 min after application, and which can be wiped off once the blood has been collected.

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Chapter 8

Humane Methods of Killing

Ethical Considerations in Killing Animals

Rearing animals for humane killing to provide tissues for studies *in vitro* represents the majority of laboratory animal usage. It can be argued that if death is not preceded by any premonition of the event, or any perception of pain, it does not affect welfare. On this basis, the reporting of statistics of animals used in experiments does not include animals reared, housed and killed by an approved method, providing no procedures were performed during life. This logic holds providing that (1) the quality of husbandry can be shown to be such that the animals have (enjoyed) a life worth living, (2) the animals' genetic background and phenotype are not intrinsically harmful and are compatible with their surroundings, (3) it is accepted that animals are not conscious of death or the potential for future life experiences and (4) death was indeed painless and free from fear.

Euthanasia, if carried out competently, may be easier for the animal than those parts of a procedure that may have caused pain, suffering or distress. On the other hand, performing euthanasia can be the most difficult part for the researcher and coping with death of research animals can take its toll on the emotions of those involved[1,2]. Killing an animal is never a pleasant task, but it does not have to be unpleasant for the animal, provided it is carried out competently and humanely. Decisions and actions on euthanasia must be made promptly if suffering is to be prevented. Collection of reliable data is essential since, without that, any suffering caused would be pointless and unethical, as the animal would have been used without purpose. For further discussion on the ethics of killing animals that have an inherent value to either themselves or others see the Nuffield Council on Bioethics (2005)[3].

Legal constraints

Killing of animals for scientific purposes is controlled by Schedule 1 to the Animals (Scientific Procedures) Act 1986 and Annex IV to EU Directive 2010/63.

When to kill an animal

Well-designed experiments should detect early signs of distress, allowing the definition of a point at which adequate data have been obtained, but any animal suffering is minimised[4]. Ideally the maximum achievable information should be obtained from each animal while suffering and distress are kept to the minimum. The precise time at which to kill the animal must be based on accurate clinical judgement, assessing the degree of suffering against the potential loss of data. To go beyond what is required for a scientific outcome may cause unnecessary suffering and is therefore inhumane. By definition, procedures carried out under the Animals (Scientific Procedures) Act will have the potential to cause pain, suffering, distress or lasting harm, so it is necessary to address how these adverse effects might be controlled or, even better, prevented. However, many experiments will lead to the death of the animal and the continual refinement of the point of intervention by euthanasia should be sought and implemented. By defining a humane end point much potential suffering can be considerably reduced by killing the animal at an early point. The application of a defined humane end point will thus utilise a lower severity level than would be needed if the procedure were allowed to run its full course. Ideally the maximum achievable information should be obtained from each animal while suffering and distress are kept to the minimum. Utilising this concept of the humane end point will refine the procedure and reduce the costs to the animals, improving the justification and the harm/benefit equation for the experiment.

The idea behind the humane end point is to define a set intervention point which allows the collection of quality scientific data but limits the amount of suffering, either contingent or direct[5], to which the animal may be subjected. The end point may be purely temporal; for example, the animal will be killed *x* hours/days/weeks after a particular technique is carried out when the adverse effects are not expected to increase but a time limit is set nonetheless, thus reducing the potential for contingent suffering.

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Or there may be a defined measurable point relating to a physiological parameter; for example, when the blood glucose level reaches *x* mmol/l, which indicates that the animal is in the required physiological state to gather the data, but not yet experiencing deteriorating clinical signs. Or, in some cases, the defined intervention point may be based on a collection of scores cumulatively measured, sometimes with weightings for more challenging clinical conditions, to ensure that any potential suffering is limited to the minimum consistent with obtaining satisfactory data. The use of a welfare assessment grid can be helpful in balancing some of the difficult decisions about the future of an animal. Such a grid can be used to give a schematic representation of parameters including clinical condition, psychological well-being, behaviour and environmental conditions[6,7]. It is unusual for laboratory animals to reach death in a laboratory as an expected consequence of a procedure without the intervention of euthanasia. For further detail on assessing welfare to aid with defining humane end points see Chapter 4 in this volume.

Decisions on euthanasia must be reached swiftly and appropriate actions taken promptly if suffering is to be prevented. Euthanasia is defined by the *Oxford English Dictionary* as the bringing about of a gentle and easy death, so it should not in itself cause pain or suffering. Experimental animals may need to be killed for a variety of reasons. The Animals (Scientific Procedures) Act requires that at the end of a series of regulated procedures the animals used must be killed, except in particular circumstances. There are three possible end points to every experiment, as follows.

1. Experimental end point: the experiment has simply run its course, and adequate experimental data have been collected.

2. Error end point: sometimes mistakes happen, there is not necessarily a welfare impact but it may invalidate the experiment and therefore it has to be ended prematurely; there is no point in continuing since any data generated will be erroneous,

3. Humane end point: the animal is killed at a defined point to limit its suffering. Waiting until the animal dies should be avoided as the end point for animal experiments. Use should be made of defined alterations to the animal's usual state, such as behavioural changes, changes in body temperature, body condition or weight loss.

In addition, any animal that is suffering severe pain, which cannot be alleviated, must be humanely killed immediately. Animals may also be killed if their health gives cause for concern, if they have reached the end of their breeding life, if they are unwanted stock, or if tissues and blood are required.

Euthanasia is frequently the most difficult part of an experiment for the researcher, particularly if the animal has been used on a long-term project and is one of the higher species. By becoming a personal licence holder you must accept that you are responsible for taking that animal's life, and feeling compassion is a necessary part of that responsibility. Whichever method of humane killing is chosen, it is very important that the operator is confident and competent to carry it out swiftly and humanely. However, if there is any hesitancy, animals may experience unnecessary distress. No one should be expected to carry out a method of humane killing with which they are not comfortable. So, if in doubt, don't do it.

The definition of death is that an animal shall be regarded as continuing to live until the permanent cessation of the circulation or the destruction of the brain; therefore, any method of killing used must ensure that one or both of these criteria is met. Legislation imposes restrictions on the methods of killing which may be employed for experimental animals, or stock animals that are surplus to requirements. Schedule 1 to the Act lists standard methods of humane killing (see Table 8.1). Killing an experimental or a stock animal using a method listed in Schedule 1 for that particular type of animal is not a regulated procedure, so neither a project nor a personal licence is required to carry this out. However, the person carrying out the killing must be competent to do so without causing distress to the animals involved. Therefore training and competence must be ensured by local management and a register of those deemed competent should be kept and regularly updated. If a method of killing is used which is not in Schedule 1, or if one of the listed methods is used for an animal for which it is not deemed appropriate, then it becomes a regulated procedure, and must be authorised on appropriate licences.

Table 8.1 Schedule 1: appropriate methods of humane killing.

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1. Prior sedation or anaesthesia may be used for any method specified in Table 8.1 a if it is in the welfare interest of the animals concerned and the method is applied by the designated veterinary surgeon or under their direction by a trained and competent person.
2. Subject to paragraph 3, the methods of humane killing listed in both tables a and b are appropriate for the animals listed in the corresponding entries in those tables only if the process of killing is completed by one of the methods listed in sub-paragraphs (a)–(f):
   (a) confirmation of permanent cessation of the circulation,
   (b) destruction of the brain,
   (c) dislocation of the neck,
   (d) exsanguination,
   (e) confirming the onset of rigor mortis,
   (f) instantaneous destruction of the body in a macerator.
3. Paragraph 2 does not apply in those cases where Table 8.1a specifies one of the methods listed in that paragraph as an appropriate method of humane killing.

(a)

Methods for animals other than fetal, larval or embryonic forms

Method	Animals for which appropriate
1. Overdose of anaesthetic using a route and an anaesthetic agent suitable for the size and species of animal	All animals
2. Exposure to carbon dioxide in a rising concentration	Rodents and birds up to 1.5 kg. Not to be used for neonatal rodents.
3. Dislocation of the neck. Birds over 250 g, rodents over 150 g and rabbits over 150 g shall be sedated prior to cervical dislocation unless the handling required to restrain the animal for the sedation, or the method of sedation itself, will cause significantly more distress to the animal than the killing method alone.	Rodents up to 500 g Rabbits up to 1 kg Birds up to 1 kg
4. Concussion/percussive blow to the head	Rodents and rabbits up to 1 kg Birds up to 250 g Amphibians and reptiles up to 1 kg (with destruction of the brain before the return of consciousness) Fishes (with destruction of the brain before the return of consciousness)
5. One of the recognised methods of slaughter set out below which is appropriate to the animals and performed by a registered veterinary surgeon, or, in the case of paragraph (ii) below, performed by the holder of a current licence under the Welfare of Animals (Slaughter or Killing) Regulations 1995. (i) Destruction of the brain by free bullet using appropriate guns, rifles and ammunition, or (ii) Captive bolt, percussion or electrical stunning followed by destruction of the brain or exsanguination before return of consciousness	Ungulates

(b)

Methods for fetal, larval and embryonic forms

Method	Animals for which appropriate
1. Overdose of anaesthetic using a route and anaesthetic agent appropriate for the size, stage of development and species of animal	All animals
2. Refrigeration, or disruption of membranes, or maceration in apparatus approved under appropriate slaughter legislation, or exposure to carbon dioxide in near 100% concentration until they are dead	Birds Reptiles
3. Cooling of fetuses followed by immersion in cold tissue fixative	Mice, rats, rabbits
4. Decapitation	Mammals and birds up to 50 g

A Section 2C licence includes conditions requiring the holder to keep a register of people who are competent to kill protected

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animals. In relation to each person named it must specify the description of animal and the methods of killing that the person is competent to use for that type of animal. A Section 2C licence also includes conditions for ensuring that a person is not registered unless (s)he has been adequately educated and trained in the killing of animals; that they are supervised when killing animals until they have demonstrated the required competence; and that at all times there is an adequate number of registered people present to enable the expeditious killing of any protected animal at that institute that needs to be killed.

Preparation for humane killing

As with any regulated procedure, humane killing requires a certain amount of preparation. Points to be considered are outlined here.

- Practice: any method of killing can cause distress if badly performed, so staff must be suitably trained and competent in the methods they will be using. Humane killing is included in accredited training courses (see Chapter 1).
- Handling: animals must be handled carefully and competently without causing them distress (see Chapter 5), until unconsciousness has occurred. Staff must be trained and competent to hold animals properly and securely. Some methods of killing may require two or more people to hold the animal, so make sure that there are sufficient people available to do the job properly. Animals will often be calmer if held by a person with whom they are familiar.
- Location: if an animal is frightened but conscious it may exhibit total immobility or it may show behavioural responses such as vocalisation, struggling, urination, defaecation, anal sac emptying and muscle tremor. Any fear or distress experienced can be communicated by sound or smell to other animals causing distress. Some of these responses may even be exhibited by unconscious animals before death occurs. It is most important that animals are removed to another room away from the group before they are killed.
- Equipment: some methods of killing require equipment such as anaesthetic-induction chambers, cylinders of gases, bottles of anaesthetic agents, needles and syringes, or firearms. It is essential to make sure that any equipment is clean, prepared and ready for use at the start, and that the operator is fully trained and competent to use it.

Methods of Euthanasia

There are a number of different methods of euthanasia available. There are a variety of guidelines that can provide assistance with the considerations relating to the carrying out of euthanasia[8–14]. The following points should be considered when choosing a method of humane killing[15,16]:

- death must occur without producing pain;
- the time required to produce loss of consciousness must be as short as possible;
- the time required to produce death must be as short as possible;
- the method must be reliable and non-reversible;
- there must be minimal psychological stress on the animal;
- there must be minimal psychological stress to the operators and any observers;
- it must be safe for personnel carrying out the procedure;
- it must be compatible with the requirements of the experiment;
- it must be compatible with any requirement to carry out histology on the tissues;
- any drugs used should be readily available and have minimum abuse potential;
- the method should be economically acceptable;
- the method should be simple to carry out with little room for error.

The different techniques are often divided into those using physical methods and those using chemicals administered by various routes. Some of the commonly used methods available are as follows.

Chemical methods of euthanasia

Overdose of anaesthetic agent

An overdose of anaesthetic, using a route and agent suitable for the species and size of animal, is considered to be suitable for all

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animals. With modern anaesthetic agents and combinations used to achieve balanced anaesthesia (see Chapter 9) there is generally a fairly wide safety margin, and indeed with some anaesthetics it is actually quite difficult to kill an animal using a reasonable volume or concentration. When carrying out euthanasia it is therefore important to select the agent carefully. The aim is to cause rapid loss of consciousness followed by respiratory depression, leading to hypoxia and death.

By injection

The drug of choice is pentobarbitone, which does not have a wide safety margin and so is unsuitable as an anaesthetic agent, but is suitable for euthanasia. It is usually presented as a 20% solution (200 mg/ml) and is administered at a dose of at least 140 mg/kg. It is preferable to give it intravenously for the most rapid action. If it is not possible to locate a vein in a conscious animal, it may be necessary to sedate it first with an agent administered by an easier route. When the sedative has taken effect and the animal can be handled more easily the pentobarbitone can be administered intravenously to kill it quickly and humanely. In the smaller species it may be administered intraperitoneally, using a suitably sized needle (see Chapter 7). The smaller the needle the better, as it will cause less pain on insertion, but pentobarbitone causes pain on injection and combination with a local anaesthetic agent will help to reduce this[17]. Pentobarbitone must not be given via the intramuscular or intrathoracic routes as it is very irritant to the tissue and will be painful. Intracardiac injection of pentobarbitone should only be attempted after the animal has been rendered unconscious.

By inhalation

Euthanasia can be carried out by exposing the animal to a high concentration of an inhaled anaesthetic agent, such as isoflurane. This is done in an induction chamber, with the agent piped in from an anaesthesia machine to ensure the even distribution of vapour. Depending on the agent chosen and the concentration used, unconsciousness usually occurs quite rapidly but death may take rather longer. It is important that the animal is physically separated from the liquid agent since volatile anaesthetics are very irritant to mucous membranes. While inhalation anaesthetics may be less aversive than carbon dioxide, they are still not particularly pleasant. Halothane has been found to be least aversive for rats and enflurane for mice[18], although such agents should be used with appropriate scavenging systems to ensure compliance with health and safety regulations. It has therefore been suggested to use halothane (although this agent has been withdrawn from some markets) to induce loss of consciousness, then to infuse carbon dioxide to cause death. It is important to ensure that the animal is left in the chamber for long enough, and to confirm that it is dead, since it may recover if it is removed too soon and allowed to breathe room air. The chamber should be designed so that the animal can be easily observed, and so that it can be easily cleaned between batches of animals. Rodents communicate by pheromones in urine and faeces and placing the animals on disposable paper, which can be replaced each time, is a simple way of controlling this potential stress factor. Chambers of this type should always be used in an extraction cabinet or with a suitable ducting system to reduce exposure of staff to the volatile agent.

Similarly, as for injectable agents, modern inhalational anaesthetic agents are developed to have a wide safety margin and so it can be quite difficult to kill an animal with such agents. It may therefore be preferable to use them to induce unconsciousness and then kill the animal using carbon dioxide at 100%. This way the detection of pain from nociception will be avoided and death from hypoxia is rapid. Inhalation is suitable for small animals, but it can be difficult to restrain larger animals, and diving animals can hold their breath for long periods, making induction unacceptably slow. Newborn animals are resistant to hypoxia and can take an unacceptably long time to die by this method.

By immersion

Animals such as fishes, small amphibia and cephalopods may be euthanased by immersion in an agent such as tricaine methane sulphonate (MS222), which is then absorbed percutaneously. As for inhalation euthanasia in the rodent, care must be taken to ensure that the animal is left in the solution for an adequate length of time and that death has occurred when it is removed.

Exposure to carbon dioxide

The use of carbon dioxide for euthanasia, while very common, is controversial, leading to much debate over the most humane method of euthanasia[19,20]. It is now established that rats and mice find carbon dioxide aversive[21] and its use as a euthanasia agent is considered unacceptable by many[22]. Carbon dioxide exposure causes immediate bradycardia due to nasal nociception activation and pain, and in humans carbon dioxide inhalation is painful at 50–100% exposure concentrations[23]. If rodents are exposed to a high concentration (>50%) of carbon dioxide, the time to unconsciousness and therefore the time for which the pain has to be

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endured is at least 10–15 s20. Many guidelines for euthanasia state that the concentration of carbon dioxide should be *rising*. However, if the chamber is filled slowly it can take around 2 min to reach unconsciousness at 35% CO_2[24]. While this avoids the acute pain seen with 100% CO_2, there is significant dyspnoea and tachypnoea which may be very distressing to the animal even if it is not actually painful. Hawkins et al.20 state that carbon dioxide aversion in rats starts at a concentration of 10–15% and that this aversion is very high, not even being overcome by motivation of 24 h food deprivation. In humans, dyspnoea occurs at 7% carbon dioxide, is severe at 15–20% and said to be very unpleasant. Rats will show escape behaviour at 15–20% CO_2. The choice with carbon dioxide would therefore appear to be between a high concentration initially, resulting in a faster more painful death, or a lower rising concentration of carbon dioxide, resulting in a slower death with less pain but more distress from dyspnoea. Neither of these is satisfactory.

One of the principal advantages of this method of euthanasia is that it can be carried out with the animal in its home cage and by placing the entire cage in a carbon dioxide cabinet. It has been shown that when killing entire cages of animals it is advantageous to kill them in the home cage as this causes much less stress[25] than removing them to an unfamiliar location. The animal is not handled and the operator is able to remain detached from the process of killing.

Exposure to a rising concentration of carbon dioxide causes animals to become unconscious due to a direct narcotic effect of the carbon dioxide then, as the concentration increases, they die from hypoxia. The concentration of carbon dioxide used for euthanasia must be rising and the animals should be placed in a chamber containing room air that is easy to clean and gives a clear view of the animals inside. It is important that the chamber is not overcrowded. The flow of carbon dioxide should then be turned on and gradually increased, displacing the air. After use the chamber must be inverted to tip out all the residual carbon dioxide since it is heavier than air and will sink to the bottom of the chamber. A controllable source of carbon dioxide should be used; it is not acceptable to use dry ice and preferably there should be a thermostatic mechanism to ensure that the gas reaching the animal from the cylinder is not uncomfortably cold.

Numerous studies in animals have indicated that carbon dioxide is aversive to breathe (see for example Gregory et al.[26] and Leach et al.21), and animals killed by this method frequently exhibit epistaxis and damage to the nasal mucosa. If animals huddle together some animals may be held in pockets of air and avoid the gas, therefore remaining alive or suddenly being exposed to high concentrations. Unintended recovery after apparent death from carbon dioxide has also been reported, so death must be confirmed by exsanguination or dislocation of the neck. If carbon dioxide is to be used effectively and humanely it must be used properly. There should be standard operating procedures in place, which must be followed.

Neonatal animals and cold-blooded vertebrates are resistant to hypoxia, and diving birds and mammals can hold their breath for long periods, so the use of carbon dioxide is not recommended in these animals. The use of carbon dioxide for stunning or for euthanasia in farm animals has also been shown to be aversive whereas combination of the carbon dioxide with argon is less so in pigs[26,27] and chicks[28]. The use of argon raises health and safety concerns since it is difficult to detect and rats and mice still show some aversive behaviour, despite it being less so than with carbon dioxide[18].

The question is therefore whether there are any better methods that combine the advantages of not having to handle the animal and the aesthetic acceptability of being remote from the animal at the point of death, but which does not cause the animal respiratory distress or pain. While carbon dioxide can be a quick and simple method for the euthanasia of large numbers of animals, practical considerations should not take precedence over animal welfare, and alternative methods may be more humane.

Physical methods of euthanasia

Physical methods should cause immediate loss of consciousness through physical trauma to the brain. If carried out properly they are often more humane than some chemical methods, since unconsciousness is achieved very quickly. However, they may be distasteful to the person carrying them out, and this leads to a tendency to be rather hesitant. However, human feelings should not be allowed to influence the choice of the most humane techniques and manual dexterity and an ability to handle the animal confidently are essential to minimise any apprehension. It is essential to make careful observations of the method being carried out by a competent and experienced person, then to practise on dead animals before carrying out these methods on live animals. Death should be confirmed by exsanguination through the severing of major vessels or by destruction of the brain.

Dislocation of the neck

If carried out correctly dislocation of the neck causes extensive damage to the brainstem and instantaneous unconsciousness. Dislocation must be carried out quickly and with confidence, otherwise there might not be complete separation of the cervical vertebrae and the animal may experience distress or pain. It may be preferable to sedate or anaesthetise animals prior to cervical

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dislocation.

For small rodents, put the animal on a surface which it can grip, place a pencil or similar object firmly across the back of the neck, take a firm grasp around the hindquarters or the base of the tail and pull firmly. The neck will be dislocated and the animal will die instantly. For larger rodents and lagomorphs, hold the body firmly in one hand, the head in the other and pull firmly with a rotating action. For larger birds, hold the legs or body in one hand, and pull the neck firmly down and backwards with the other. It may be necessary to wrap the bird to prevent involuntary flapping of the wings, and to confirm death by severing the major vessels in the neck.

Concussion

Concussing the animal by striking the back of the head renders the animal unconscious, following which death must be ensured by dislocation of the neck or by exsanguination. As with dislocation of the neck, confidence in handling the animal and manual dexterity are required to carry out this method without causing distress to the animal. Support the animal by the hindquarters and swing the body downwards such that the back of the head comes sharply into contact with a hard surface such as a work bench. Considerable training and practice on dead animals is required to ensure competence with this method, and it is difficult to ensure that animals are stunned consistently. For amphibia, reptiles and fishes the brain must be destroyed immediately following concussion, as in these species the brain is very tolerant to hypoxia, and it cannot otherwise be guaranteed that concussion is irreversible or that unconsciousness will last until death.

Other methods of euthanasia

The physical methods described above are appropriate for small rodents, rabbits and birds and also for amphibians, reptiles and fishes. For ungulates, the animal may be killed by one of the standard methods of slaughter, such as use of a free bullet, a captive bolt or electrical stunning followed by destruction of the brain or exsanguination. However, these methods are controlled by other legislation, including control of firearms and will therefore only be available to those who have been appropriately trained and issued with the relevant licences.

For fetal, larval and embryonic forms there are other methods that can be used which take into account the degree of development of the nervous system in the various animals. Overdose of injectable anaesthetic may be used for all species. Refrigeration, disruption of membranes or maceration can be used for birds and reptiles, but the death of the reptile embryo must be ensured by overdose of anaesthetic, maceration or immersion in tissue fixative. Cooling of fetuses until movement has stopped followed by immersion in cold tissue fixative is appropriate for mouse, rat and rabbit fetuses. Decapitation using a sharp implement can be used for fetal mammals and birds up to 50 g in weight. In some jurisdictions decapitation for neonatal rodents is considered an appropriate method of euthanasia.

Other methods of killing may be employed occasionally if there is a scientific need. These will require authority on both personal and project licences. Microwaves can be used to fix brain metabolites without losing anatomical integrity[29,30]. Specialist apparatus is required, along with careful technique. The microwaves are focused on particular areas of the brain, producing death very rapidly. Electrical stunning may be used as in routine pre-slaughter stunning for ungulates and also for other species such as poultry or rabbits. It requires specialist equipment, and death must be confirmed using another method. Pithing may be carried out in unconscious fish, amphibia or reptiles. A sharp needle is inserted through the foramen magnum and agitated to destroy the brain. This requires technical skill to ensure rapid death, and must not be carried out in conscious animals. Rapid freezing using liquid nitrogen can be used *in situ* or following decapitation to freeze the brain. The animal must be rendered unconscious first, as it can take up to 90 s to freeze deep structures. Decapitation may not be humane in conscious animals, because of the length of time it takes for the decapitated head to lose consciousness, particularly in cold-blooded vertebrates. If this method is required for scientific reasons there must be particular justification, and consideration given to sedating or anaesthetising the animal first.

Schedule 1 methods

Schedule 1 is in two parts (see Table 8.1). Table 8.1a describes methods suitable for animals other than fetal, larval and embryonic forms, and Table 8.1b methods for fetal, larval and embryonic forms. Note that killing pregnant animals is usually considered to kill the fetuses, so killing a dam by a Schedule 1 method would not require licence authority. However, the subsequent use of any live fetuses more than two-thirds of the way through gestation for scientific study would require authority. Newborn animals fall under the first definition.

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A: animals other than fetal, larval and embryonic forms

Overdose of anaesthetic by injection, inhalation or immersion

An overdose of anaesthetic, using a route and agent suitable for the species and size of animal, is considered to be suitable for all animals.

Exposure to carbon dioxide

Unconsciousness occurs more rapidly in smaller animals, so Schedule 1 restricts the use of carbon dioxide to the killing of rodents and birds up to 1.5 kg in weight.

Physical methods

Dislocation of the neck

This method is appropriate for rodents up to 500 g, rabbits up to 1 kg and birds up to 1 kg.

Concussion

Concussion may be used for rodents and rabbits up to 1 kg, birds up to 250 g and amphibians and reptiles up to 1 kg and fishes.

Recognised methods of slaughter

Ungulates may be killed under Schedule 1 by one of the standard methods of slaughter, such as use of a free bullet, a captive bolt or electrical stunning followed by destruction of the brain or exsanguination.

B: fetal, larval and embryonic forms

The Animals (Scientific Procedures) Act 1986 covers immature forms from particular stages of development. For mammals, birds and reptiles this is two-thirds of the way through gestation or incubation, and for amphibia, fish and cephalopods the point at which they become capable of independent feeding. Schedule 1 describes methods of humane killing that can be used for these forms, which take into account the degree of development of the nervous system in the various animals. Some species are highly developed at birth and therefore require special consideration during the last few days of gestation or incubation, whereas others are less well developed and are less able to perceive pain at this stage.

Overdose of anaesthetic

This may be used for all animals. An anaesthetic agent appropriate for the size and stage of development of the animal should be chosen. In large fetuses or embryos anaesthetics may be given by the intravenous or intraperitoneal routes; smaller animals and larvae may be immersed in anaesthetic.

Refrigeration, disruption of membranes, maceration or exposure to 100% CO_2

These methods can be used for birds and reptiles. In birds, cooling to below 4°C is effective, but reptile embryos are resistant to hypothermia and hypoxia, so death must be ensured by overdose of anaesthetic, maceration or immersion in tissue fixative.

Cooling followed by immersion in fixative

Mouse, rat and rabbit fetuses may be chilled until movement has stopped, then immersed in cold (4°C) tissue fixative.

Decapitation

This is a humane and quick method that can be used for mammals and birds up to 50 g in weight which are not well developed, using a sharp implement. Note: this method cannot be used under Schedule 1 for neonatal mammals.

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Confirmation of death and disposal of carcasses

After carrying out any method of euthanasia it is vital to check that the animal is really dead before disposing of the carcass. Thus the process of killing must be completed by one of the following six methods:

1. confirmation of permanent cessation of the circulation, for example by severing the major vessels;

2. destruction of the brain with a permanent loss of brain function;

3. dislocation of the neck;

4. exsanguination;

5. onset of rigor mortis;

6. mechanical disruption by instantaneous destruction of the body in a macerator.

All animal carcasses are classed as clinical waste and as such must be disposed of correctly either by maceration or in clinical waste bags that are then incinerated. There will be local rules in the work area relating to the method of disposal and these must be followed.

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Chapter 9

Anaesthesia and Analgesia

Introduction

Scientific procedures carried out on animals may cause them to experience pain or distress. These impact on the welfare of animals and may adversely affect the quality of scientific data obtained. Steps must be taken to minimise these adverse effects, to safeguard the welfare of animals, to ensure scientific validity and to comply with legal requirements. EU Directive 2010/63 requires that procedures are carried out under general or local anaesthesia, unless anaesthesia is incompatible with the experiment or more traumatic than the procedure itself, and that analgesia or another method is used to ensure that pain, suffering and distress are kept to a minimum. Anaesthesia and analgesia in laboratory animals is a rapidly expanding discipline and regular review of anaesthetic and analgesic practices is required to ensure the most up-to-date methods are being employed.

Anaesthesia can be defined as a local or general loss of sensation, with or without the loss of consciousness, brought about by controlled reversible intoxication of parts of the nervous system.

- *General* anaesthesia is a state of total unconsciousness, resulting from the administration of anaesthetic drugs that depress the function of the brain.
- *Local* anaesthesia is a loss of sensation in a small area of the body, produced by injecting or applying local anesthetic drugs, which block nerve conduction.
- *Regional* anaesthesia is a loss of sensation in a region of the body produced by application of a local anaesthetic agent to all the nerves supplying that region; for example, epidural anaesthesia.

Clearly it is essential to induce and maintain general or local anaesthesia if a potentially painful procedure is to be carried out on an animal, and also occasionally it may be necessary to use sedation or anaesthesia to restrain an animal for a procedure which may not be painful, but which may cause distress to the conscious animal.

The main purpose of anaesthesia is to produce humane restraint and an absence of pain, but in addition anaesthesia must be safe for both animal and operator, produce consistent and repeatable results and be easy and practical to administer. However, successful anaesthesia is not achieved simply by administering a set drug or combination to an animal: the way an animal responds to anaesthesia can be unpredictable and depends on numerous factors, some of which are under the control of the operator, and some of which are not. These must be taken into account when designing an anaesthesia regime, otherwise the procedure may not be successful, resulting in unnecessary pain or distress for the animal, prolonged recovery times or even death of the animal.

Good anaesthesia requires more than just choosing the right agent(s). A good standard of animal care must be maintained, before during and after anaesthesia, to reduce stress and provide control of pain and distress. Lack of attention to care of the animal in the pre- and post-anaesthetic periods is a common failing of laboratory animal anaesthetic practice[1]. Problems, however, can be avoided by good pre-, intra- and post-anaesthesia care.

Pre-Anaesthetic Care and Evaluation

There are a number of factors to be considered in the pre-anaesthetic period.

Acclimatisation

It is important for animals to become acclimatised to their environment prior to the induction of anaesthesia. Animals become stressed during transport, and normal physiological responses to this stress will result in abnormal experimental measurements. Mice may need at least 48 h after transportation by road or aeroplane for high corticosterone levels to return to normal, and may

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need 1–2 weeks to acclimatise fully[2]. Even transporting animals between animal rooms can result in stress, requiring 4 days or more to resolve[3]. It is important to plan experiments such that animals have time to settle in before being subjected to procedures.

Training

Many animals can be trained to accept the handling required to facilitate procedures. Most animals can become accustomed to handling, and some can be trained to present themselves for injection or sampling. The temperaments of individual animals vary and some will be more suited to certain types of procedure than others, so choosing animals carefully to ensure they are suited to the procedure can reduce the stress placed on the animals.

Fasting

Fasting prior to anaesthesia is required in some species, to prevent vomiting on induction or recovery, or reflux of stomach contents into the oesophagus during anaesthesia. Many laboratory animals, including rodents and rabbits, cannot vomit, and are prone to the development of hypoglycaemia if deprived of food. These animals need only be fasted if there is an experimental reason for doing so, such as for upper gastrointestinal tract surgery, but in any case the stomach will only be empty if coprophagy is prevented. Birds have a high metabolic rate, and small birds should not be fasted for more than 2 h. Large birds (e.g. ducks or chickens) may be fasted for 6–12 h to reduce the risk of regurgitation. Fasting in reptiles and amphibians is generally not necessary.

Single-stomached animals, such as dogs, cats, ferrets, pigs and non-human primates, vomit readily, and these animals should be fasted for 8–12 h before anaesthesia. Fasting in ruminants has very little effect on the volume of food in the stomach, but can reduce the risk of bloat. Fish should be fasted for 24–48 h.

Handling

Careful and *expert handling* of the animals is important. Firm but sympathetic handling by a familiar handler can reduce the stress experienced by the animals in the pre-anaesthetic period.

Health status

It is essential to use animals that are healthy and disease-free. These are less likely to have problems during anaesthesia than those with overt or subclinical disease. Those with respiratory infections in particular should be avoided. It is best to use animals of known health status (see Chapter 6), but this may not be possible, and sometimes a previous experimental procedure may have altered the health of the animal. Either way, animals should be given a *clinical examination* prior to anaesthesia to rule out infectious and non-infectious diseases. The thoroughness of the examination depends on the species, the procedure and the likely duration of anaesthesia. As a minimum, the function of the respiratory and cardiovascular systems and food and water intake should be evaluated and veterinary advice sought in the event of any deviation from normal.

Local anaesthetics

When intravenous agents are to be used for induction, local anaesthetic creams can be applied topically over the site of venipuncture. These desensitise the skin, so the animal doesn't feel the needle. They should be applied at least 40–60 min before injection[4].

Premedication

Pre-anaesthetic medication is often used in larger animals, where sedation aids induction of anaesthesia. However, pre-anaesthetic drugs may also be beneficial in small laboratory animals. The objectives of premedication include1:

- to decrease *fear* and *apprehension*, to aid in stress-free induction,
- to *reduce the amounts* of other anaesthetic agents required to induce general anaesthesia, so reducing their undesirable side effects,
- to assist a *smooth recovery* from anaesthesia,

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- to *reduce salivary and bronchial secretions*,
- to block the *vaso-vagal reflex*, in which bradycardia occurs due to endotracheal intubation and handling of the viscera,
- to *reduce post-operative pain*.

There is evidence that production and release of stress-related substances produce pain, hypersensitise the nervous system to otherwise non-painful stimuli and promote inflammation, all of which delay wound healing and depress the immune response[5]. In addition, analgesics are more effective if given before pain is perceived[6,7,8]. Premedication therefore can reduce anxiety and reduce the perception of pain following surgery.

Pre-anaesthetic drugs fall into three main categories: sedatives and tranquillizers, analgesics and anticholinergics.

Tranquillizers and sedatives

Sedatives relieve anxiety and cause drowsiness. Tranquillizers remove anxiety without causing drowsiness, but animals may still be readily roused. However, many drugs fall into more than one classification, the difference depending on dose and the species. All of these drugs potentiate the action of anaesthetics and narcotic analgesics, so are useful in calming the animal before induction of anaesthesia and in reducing the dose of other drugs required to produce surgical anaesthesia.

Benzodiazepines

Examples of benzodiazepines include *diazepam* and *midazolam*. They cause sedation, with considerable species variation. In some species (e.g. rabbits, rodents)1 they cause marked sedation, together with good skeletal muscle relaxation, and they are potent anticonvulsants. They have minimal effects on the cardiovascular system. They potentiate the action of many anaesthetics and narcotic analgesics and are a valuable adjunct to many anaesthetic regimes.

α_2-Agonists

Examples of α_2-agonists include *xylazine* and *medetomidine*. These drugs act on α_2-adrenoceptors and produce dose-dependent sedation. They are used alone for sedation, or for premedication, as they markedly reduce the quantity of anaesthetic required. Following administration there is initial hypertension, then marked bradycardia with moderate hypotension. Hyperglycaemia and polyuria also occur. Xylazine produces potent depression of the central nervous system (CNS), leading to sedation, narcosis, unconsciousness and eventually general anaesthesia with increasing doses. Its main use is in combination with ketamine to produce general anaesthesia. Medetomidine is a very potent and specific α_2-agonist that produces sedation, analgesia and bradycardia. It also causes vasoconstriction in the periphery so blood pressure is maintained despite the fall in heart rate although it may decrease slightly after a few hours. Respiratory rate may also fall slightly. Medetomidine can cause heavy urination, so care must be taken to prevent the animal becoming wet, hypothermic or even dehydrated. Cyanosis may be apparent, with venous blood being very dark. Despite this, blood oxygen saturation remains high. Medetomidine is synergistic with ketamine and opioids, such as fentanyl, and it markedly reduces the dose of inhaled agents required. Medetomidine has a specific reversal agent, *atipamezole*. This is an α_2-antagonist, which can also be used to reverse xylazine. The animal wakes up a few minutes after administration, avoiding potential problems from a prolonged recovery.

Ketamine

This is a dissociative analgesic agent which produces immobility in most species. However, it produces an increase in muscle tone and variable analgesia. Recovery can be prolonged and it may be associated with hallucinations[9]. For these reasons it is rarely used alone in most species. It can be used for premedication or sedation in Old World non-human primates.

Phenothiazines and butyrophenones

Examples of phenothiazines and butyrophenones include *chlorpromazine* and *fluanisone* respectively. These are potent sedative drugs with no analgesic properties which potentiate the action of anaesthetics. However, they produce hypotension and depress thermoregulatory mechanisms, leading to hypothermia. Therefore they are infrequently used alone. They are most frequently used as components of neuroleptanalgesia (see below).

Analgesics

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Narcotic analgesics (opioids) are potent analgesic and sedative drugs which are used to provide sedation and analgesia prior to induction of anaesthesia, and also for post-surgical pain relief. Opioid analgesics interact at opioid receptors in the CNS and other tissues. There are three main receptor types: μ (mu) or OP3, κ (kappa) or OP2 and δ (delta) or OP1. Most of the clinically used opioids interact with the OP3 and OP2 receptors, producing analgesia and sedation, but also undesirable side effects such as respiratory depression, miosis (pupillary constriction), reduced gastrointestinal motility and euphoria. These drugs are potentially addictive, so most of them are controlled drugs under the UK Misuse of Drugs Regulations 2001. Lower doses cause depression of the CNS, but high doses cause excitement. Overdoses cause coma and death. Differential actions at each receptor type lead to different effects of the various analgesics. Agents may be pure agonists, activating the receptors (e.g. *morphine*, *fentanyl* and *alfentanil*, which are pure μ agonists), mixed agonist/antagonists (e.g. *butorphanol*, which antagonises μ receptors but is an agonist at κ receptors) or partial agonists (e.g. *buprenorphine*, which has analgesic effects at μ receptors but antagonises pure μ agonists). Increasing the dose of a pure agonist increases the analgesia produced, but also increases the undesirable effects. The respiratory depression produced by the partial agonists is limited and these drugs are less liable to abuse. Partial agonists can be used to antagonise the side effects of pure agonists, but maintain analgesia. This is sometimes known as sequential analgesia. Buprenorphine produces good sedation and analgesia, although it has a slow onset of action, but it lasts for 8 h. It is useful for producing sedation, alone or with phenothiazines, as part of premedication, but is used primarily for analgesia. It is the opioid analgesic of choice for most laboratory species.

Naloxone is a pure opioid antagonist, having no sedative or analgesic action but capable of reversing the effects of an agonist.

Anticholinergics

Anticholinergic drugs were widely used in the past to reduce the production of salivary and bronchial secretions, reducing the risk of airway blockage. This was particularly important with irritant volatile agents such as ether. Modern volatile anaesthetic agents are not irritant, and the routine use of drying agents is no longer considered necessary[10]. They have a number of undesirable side effects, including increased heart rate, mydriasis (pupil enlargement) and reduced tear production. They reduce the watery component of saliva, rendering it more viscous, especially in ruminants, and decrease ciliary activity. They also reduce gut motility and sphincter tone. Their use may be appropriate if bradycardia is present, for example due to vaso-vagal reflexes triggered by handling of the viscera during surgery, or following the administration of vagomimetic drugs. *Atropine* is a natural alkaloid that crosses the blood–brain barrier and is eliminated mostly in urine. Rats, rabbits and possibly ruminants eliminate the drug rapidly. Cardiovascular effects last 40–90 min. *Glycopyrrolate* does not cross the blood–brain barrier and may be more appropriate for neurological studies, although it is slower in onset. Cardiovascular effects last 2–4 h.

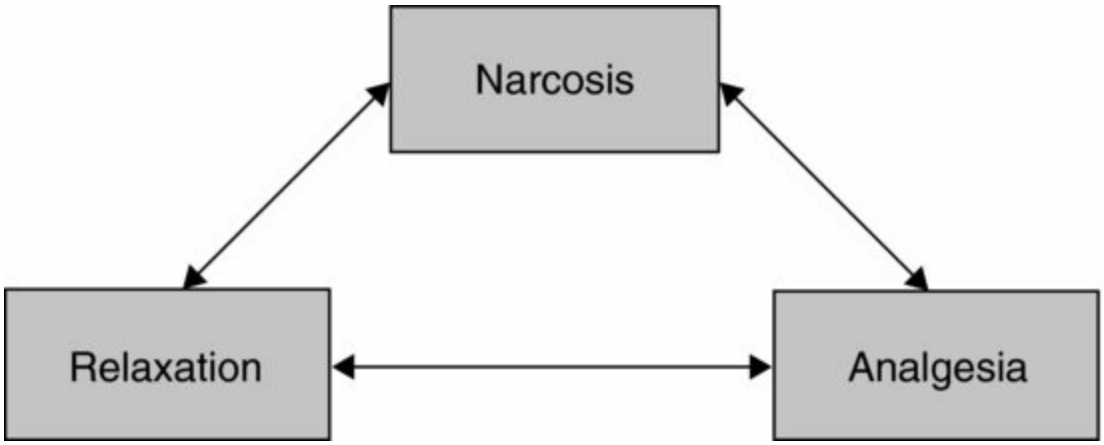
General Anaesthesia

General principles

The primary reasons for giving an anaesthetic are to provide humane restraint. This requires modification of the animal's behaviour and level of consciousness by *narcosis*, providing sufficient *muscle relaxation* to facilitate procedures and, most importantly, sufficient *analgesia* to prevent the animal experiencing pain. These three elements make up the classical triad of anaesthesia (see Figure 9.1).

FIGURE 9.1 The triad of anaesthesia.

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The ideal general anaesthetic agent has the following properties:

- provides humane restraint,
- is consistent and reliable, having the same effects each time it is used,
- has a wide safety margin, and is safe for both animal and user,
- does not interfere with the procedure or affect the scientific objectives,
- any alterations due to anaesthesia need to be minimal and consistent, to reduce any variability,
- keeps the animal physiologically stable,
- is cleared rapidly, so the animal recovers and returns to physiological normality quickly.

The production of analgesia is the single most important drug effect.

Balanced anaesthesia

No single agent alone will provide satisfactory narcosis, muscle relaxation and analgesia. This is therefore usually achieved by *balanced anaesthesia*, which is the administration of drugs in combinations, including premedicants, inhalation and injectable anaesthetics and analgesics, in order to produce stable anaesthesia and to reduce the potential side effects of any individual drug. Using several anaesthetics together can overcome many of the disadvantages encountered when using agents individually. Many anaesthetic drugs produce a synergistic (supra-additive) effect when combined, while decreasing the probability and seriousness of side effects.

Balanced anaesthetic regimes allow for minimal interference with the animal's physiology, and a smooth, rapid and pain-free recovery. Administering sedatives prior to induction or inducing anaesthesia with an injectable agent avoids the stress otherwise caused by induction with inhaled agents. Some injectable agents produce little analgesia alone, and addition of a low concentration of an inhaled agent can provide this. Similarly, when using inhalation anaesthesia, the concentration required to produce surgical anaesthesia can be reduced by administering a potent analgesic such as fentanyl. Induction of anaesthetic can be carried out using injectable combinations, followed by inhaled agents for maintenance. In large animals, the administration of an injectable anaesthetic to induce anaesthesia is essential, as it is difficult to restrain these animals for induction by a facemask. Maintenance of anaesthesia can then be satisfactorily achieved by intubation and the administration of a volatile anaesthetic.

The combination of drugs selected to produce ideal anaesthesia should include a narcotic, a behaviour modifier, a muscle relaxant, an analgesic and a stress reducer.

Choice of anaesthetic

The agents chosen to achieve balanced anaesthesia for a particular procedure will depend on a number of factors, including:

- the species,
- the duration of the procedure,
- the depth of anaesthesia required,
- the purpose of the investigation,
- the equipment available,
- the experience of the staff.

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Anaesthetic regimes of different durations and depths will be required for different procedures, and some anaesthetic drugs will be contraindicated in certain protocols. Therefore, advantage should be taken of the wide variety of anaesthetic drugs and combinations available to find one which is best suited to the experimental protocol.

Surgical techniques and anaesthesia have effects on the physiology of the animal, which can be prolonged. Surgery results in a significant stress response with corticosteroid release, and levels can remain high for several days. This is designed to assist the animal in overcoming injury, but may be undesirable in the experimental subject. The physiological effects of anaesthesia may be quite minor compared with those produced by the experimental surgery, but nonetheless an anaesthetic regime with minimal potential for adverse effects should be selected. Good anaesthetic practice is an important aspect of animal welfare, and is also relevant to the scientific validity of any study that makes use of the animals, since poor anaesthetic practice can produce unwanted variability.

The anaesthetic should be chosen when all factors potentially influencing the protocol have been considered. While the ideal drug does not exist at present, new preparations are in development continually and new anaesthetic protocols are constantly being evaluated. It is important to keep reviewing available literature to identify possible refinements.

Administration of anaesthetics

There are two common methods by which anaesthetics are administered: by injection or by inhalation. Each method has advantages and disadvantages (see Table 9.1). These can be used individually or combined. Injectable agents are often used for induction, with inhaled agents used for maintenance of anaesthesia.

Table 9.1 Advantages and disadvantages of injectable and inhalation anaesthesia.

	Injectable	Inhalation
Equipment required	Minimal	*Considerable*
Speed of induction and recovery	*Variable*	Rapid
Predictability	*Moderate*	High
Encumbrance during surgery	Minimal	*Moderate*
Ability to adjust depth of anaesthesia	*Difficult*	Easy
Safety margin	*Moderate*	High
Health and safety concerns from operator exposure to agents	Minimal	*Moderate*
Metabolism of agents and potential to interfere with the experiment	*Moderate*	None
Suitability for short procedures and for high frequency anaesthesia	*Low*	High
Operator skill required	*i.v., high* Other routes, minimal	Moderate
Potential environmental impact	Minimal	*Moderate*
Ease of administration	*i.v., difficult* Other routes, simple	Moderate
Risk of misadministration	*High*	Low
Ability to reverse overdose	*Low*	High
Stress on induction	i.v., Low/moderate *Other routes, moderate/high*	Moderate
Legal category	*Most are POM-V, some are controlled drugs*	Most are POM-V

i.v., intravenous; POM-V, Prescription Only Medicine–Veterinarian.
Entries in italic indicate disadvantages; those in normal type are advantages.

Inhalation anaesthesia

Agents used for inhalation anaesthesia are volatile liquids, which are vaporized by passing a gas such as oxygen through or over them. The vapour/oxygen mixture is then inhaled. They are then absorbed from the lung alveoli into the bloodstream and are predominantly excreted the same way. This means that induction and recovery from anaesthesia are quicker with inhalation than injectable agents. This is important in regaining normal physiology, to control post-operative hypothermia and fluid or electrolyte imbalance. Recovery usually occurs within 1–10 min, although if animals have been maintained for an hour or more on inhalation

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anaesthesia, full recovery can take 20 min or longer.

The major advantage of inhalation anaesthesia is the ability to adjust the depth of anaesthesia. If the anaesthesia is too light, the concentration of vapour is simply increased, and within a few breaths the depth of anaesthesia will increase. Similarly, if the anaesthesia is too deep, the concentration can be reduced and the excess anaesthetic is breathed off until the depth of anaesthesia lightens. This makes inhalation anaesthesia relatively safe compared with injectable anaesthesia. Reversal of overdose of anaesthetic is quicker and usually more successful with inhalation agents than injectable ones, so reducing mortality. Recovery can be hastened if attention is paid to controlling the depth of anaesthesia during the procedure. As anaesthesia progresses the concentration of anaesthetic can usually be reduced slightly, and a further reduction in depth can be made during suturing of subcutaneous tissues and skin, so that recovery time following completion of surgery is reduced.

Some metabolic breakdown of inhaled agents does occur, although this is minimal with modern volatile agents such as isoflurane (<1%), and there is little or no induction of liver microsomal enzymes, which occurs with some injectable agents. This makes inhalation the anaesthetic method of choice where normal metabolism is required. Inhalation anaesthesia is particularly suited to short procedures, repeated anaesthesia and procedures where rapid return to normal metabolism is essential.

The disadvantages of inhalation anaesthesia are that a considerable amount of specialised equipment is required to deliver the anaesthetic vapour to the animal. Waste gases produced are a potential health and safety hazard to the user and have to be disposed of appropriately. Inhalation anaesthetics are greenhouse gases and globally contribute the equivalent of 1 million cars to global warming each year[11].

Considerable skill is required to set up and operate an inhalation system. Despite these drawbacks, inhalation remains the anaesthetic method of choice for most procedures.

The volatile anaesthetic agents

Volatile agents are delivered to the animal from an anaesthesia machine using oxygen, or a mixture of oxygen and nitrous oxide, as the carrier. There are several volatile agents available. How these agents work remains incompletely understood.

The required concentration of volatile agent depends on the properties of the agent, not the weight of the animal. The dose an animal receives depends on the concentration of agent in the inhaled gas mixture, and its tidal volume. Therefore, heavier animals do not necessarily need a higher concentration of anaesthetic, but will receive a greater dose as they have a larger tidal volume. Each agent has a minimum alveolar concentration (MAC), which is the concentration at which anaesthesia is induced in 50% of animals (see Table 9.2).

Table 9.2 Induction and maintenance concentrations of volatile agents.

	Induction	Maintenance
Anaesthetic	concentration (%)	concentration (%)
Sevoflurane	5.0–8.0	3.3–4.0
Isoflurane		
Small animals	3.0–5.0	1.25–3.0
Large animals	4.0–8.0	1.5–3.0

Isoflurane is a volatile liquid at room temperature which vapourises readily, with a boiling point of 48.5°C. It is completely nonflammable. Isoflurane produces very rapid induction and recovery from anaesthesia, allowing the depth of anaesthesia to be altered very readily. It reduces pain sensitivity and relaxes muscles, producing all three components of the anaesthetic triad to an acceptable degree. It can be used just with oxygen to produce anaesthesia suitable for most surgical procedures, and can be combined with opioid analgesics or nitrous oxide where additional analgesia is needed.

Isoflurane undergoes no metabolism and does not affect hepatic microsomal enzymes, so is suitable for studies requiring normal liver metabolism[12]. The maximum exposure limit for personnel is 50 ppm[13].

Isoflurane causes some respiratory and cardiovascular depression, leading to a fall in blood pressure due to vasodilation. Although not irritant it has a pungent odour, which can cause some animals such as rabbits to breath-hold on induction. Although its use is declining in human medicine it is used commonly in veterinary anaesthesia.

Sevoflurane is a nonflammable liquid used for induction and maintenance of general anaesthesia. It has a boiling point of 58.6°C. Together with desflurane, it is replacing isoflurane in human anaesthesia. It is highly volatile, with a very fast induction and recovery. It is good where mask induction is needed as it is not irritant and is sweet-smelling. However, it produces profound respiratory depression in some species and there is a risk of accidental overdose due to the speed with which changes in inhaled

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concentration take effect. Sevoflurane is unstable in the presence of soda lime1 so should not be used in rebreathing anaesthetic circuits (see below). There is no workplace exposure limit defined for sevoflurane. The global warming potential of sevoflurane is lower than that of other inhalation agents.

Nitrous oxide is a colourless non-flammable gas with a slightly sweet odour. It is a weak general anaesthetic, produing euphoria on inhalation, but it is not potent enough for induction or maintenance of anaesthesia in most species. However, it has minimal cardiovascular and respiratory effects, and can be used together with a volatile agent to reduce the required concentration of that agent, reducing its side effects, while providing additional analgesia. It can be used 60/40 or 50/50 with oxygen to deliver the volatile agent for anaesthesia in larger species. It may also be beneficial in prolonged anaesthesia over 24 h, where administration of volatile agents in 100% oxygen can lead to oxygen toxicity. After prolonged anaesthesia, 100% oxygen should be given for 5–10 min, or oxygen can be displaced from the lungs by the nitrous oxide as it is breathed off, causing diffusion hypoxia, which can lead to suffocation. The occupational exposure limit for nitrous oxide is 100 ppm.

Anaesthetic machines

It is recommended that an anaesthetic machine with an appropriate vaporizer be used for inhalation anaesthesia. These allow precise control of the amount of volatile anaesthetic agent delivered to the animal, allowing precise control of the depth of anaesthesia. The components of an anaesthetic machine are:

- a source of oxygen (with or without nitrous oxide) as a carrier gas,
- a flowmeter to measure the oxygen flow,
- an anaesthetic vaporizer,
- a delivery system from the vaporizer to the animal,
- a mechanism to deal with waste gases.

Medical-grade oxygen and nitrous oxide can be provided from cylinders of compressed gas attached to the anaesthetic trolley, or piped into the facility. Where cylinders are used a *reducing valve* attaches to the cylinder to ensure that a constant low pressure of gas is delivered to the system. From the cylinder, the carrier gases pass through *flowmeters*, which allow volume of gas passing to the animal to be monitored and adjusted. The flowmeter consists of a graduated glass tube with a bobbin, which floats at a level determined by the amount of gas passing through the tube. The tube is calibrated such that the top of the bobbin is in line with the marking indicating the flow rate of the gas. The bobbin should rotate in the tube. Each flowmeter is calibrated specifically for a particular gas.

The carrier gases are then passed through a *vaporizer*, over the volatile agent. The vaporizer delivers a known concentration of the anaesthetic vapour over a given range of flow rates and temperatures. Each vaporizer is calibrated for a particular agent and should not be used with other agents.

All anaesthetic equipment must be serviced regularly to ensure the correct quantities of vapour are delivered. The machine must be checked before each anaesthetic is given to ensure the gas cylinders and vaporizer are full, there are spare cylinders, a key to turn them on is available and the flowmeters are working. The anaesthetic circuit, endotracheal tubes and scavenging system must be checked to make sure they are intact, correctly attached and functional. If any monitoring equipment is to be used it must be set up properly and in working order. Only once all these have been checked should the animal be anaesthetised.

Induction of anaesthesia

Inhalation anaesthesia can be induced in larger animals, such as pigs, using a face mask. Animals may need sedation for this, as many resent the handling required. In small animals they can be placed in an induction chamber, and oxygen containing anaesthetic vapour piped into the chamber. The chamber is a simple box with transparent sides so that the animal can be observed during the induction, and must be easy to clean. Absorbent paper can be placed on the floor of the chamber. The inlet pipe is at low level and the extract at high level, since the volatile agents are heavier than air. Anaesthesia is induced by piping in the induction concentration of the agent (see Table 9.2). As the animal breathes the anaesthetic vapour, it is absorbed in the lungs and begins to circulate. The concentration in the CNS starts low and gradually builds, until enough has been absorbed to induce anaesthesia. This can take several minutes and there is a time lag between inhaling the vapour and the onset of anaesthesia. The speed of onset depends on the concentration of vapour in the inspired gas and the particular agent used. To increase the speed of induction, the concentration of vapour in the inspired gas may be increased to a level which, if it were maintained, would cause excessive depression of the CNS and death. This is known as the induction concentration. Once anaesthesia is induced – the animal has lost its righting reflex, is immobile in the chamber and breathing regularly – the animal can be removed from the chamber. Anaesthesia will persist for a brief period (about 1 min), which may be sufficient for short procedures (such as ear punch). For longer

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procedures, anaesthesia can be maintained via a face mask or endotracheal or intranasal tube for as long as is required. The concentration of anaesthetic vapour must be reduced to a suitable maintenance concentration (Table 9.2). The concentration needed will vary with the individual animal, the nature of the procedure, and whether other drugs have been given to provide additional analgesia. The major advantage of inhalation anaesthesia is that the concentration can be adjusted to meet the exact needs of the individual animal.

After the procedure the chamber should be tipped to empty out any residual anaesthetic gas, and be cleaned between animals; otherwise pheromones left by previous occupants can cause stress to animals newly placed in the chamber.

Maintenance of anaesthesia: anaesthetic circuits

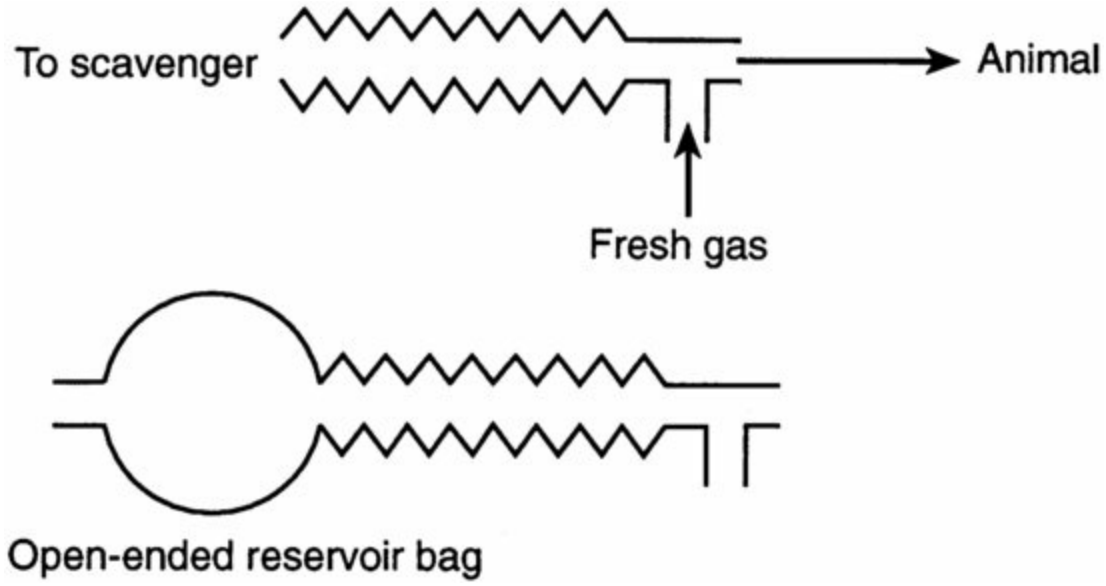
To maintain anaesthesia, the mixture of gases and volatile agent is delivered to the animal through an anaesthetic circuit. There are essentially two types of circuit: those where carbon dioxide is absorbed (rebreathing circuits) and those where it is not (non-rebreathing circuits). The particular one chosen depends on the size of the animal, the site of the operative field, whether intermittent positive pressure ventilation (IPPV) is to be used, and personal preference. The quantity of oxygen piped into the circuit depends on the needs of the animal (which depends on its body weight), and the type of circuit.

For non-rebreathing circuits, the expired air is not re-inhaled, and fresh gas from the anaesthetic machine is delivered continually to the animal. The exhaled gases are swept out of the open end of the circuit by the fresh gases flowing in during the expiratory phase. If the fresh gas flow rate is too low, rebreathing may occur, and carbon dioxide can build up to dangerous levels as there is no method of removing it. It is essential, therefore, to have a high-enough flow rate to remove the alveolar gas when it is expired so the animal does not rebreathe exhaled gases.

Most laboratory animals are small, and therefore have small tidal volumes. They also have limited capacity to continue breathing if there is resistance in the anaesthetic circuit. For these animals it is important that an anaesthetic circuit is used with low resistance to breathing and a low dead space.

T-piece circuits have minimal resistance and little dead space and are the circuits of choice for animals up to 3 kg in body weight (Figure 9.2). The open tube acts as a reservoir of fresh gas. Exhaled gases are pushed out of the open end of the reservoir tube by fresh gases flowing in from the anaesthetic machine during the expiratory phase. T-piece circuits require a high gas flow rate, at least twice the animal's minute volume, and are therefore expensive to use for larger animals.

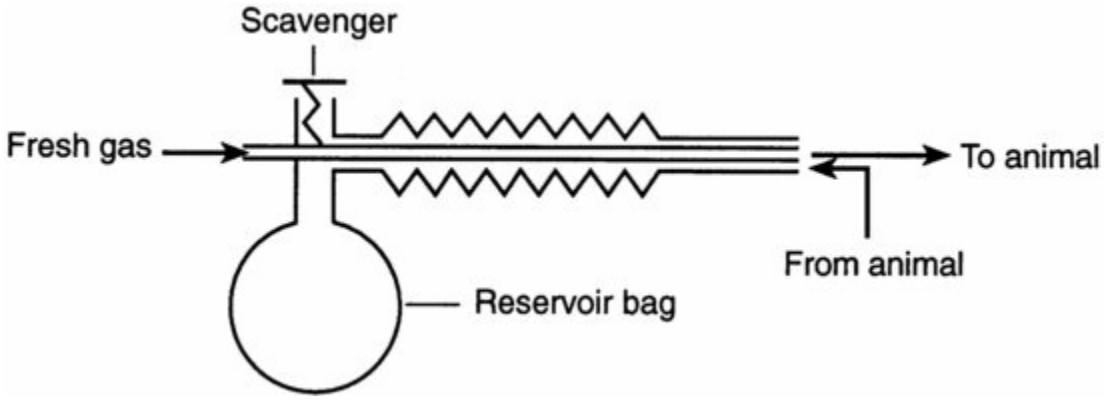
FIGURE 9.2 T-piece circuits.



Co-axial circuits are a modification of the T-piece where the fresh gas inflow pipe runs inside the reservoir limb. During expiration the dead-space gases pass down the outer corrugated tubing to the scavenger. Some co-axial circuits, for example the Bain circuit (Figure 9.3), have a valve on the expiration arm. As the system fills, the pressure increases to a level at which the expiratory valve opens and then expired alveolar gas escapes through the valve. Since the valve increases resistance to breathing these circuits cannot be used with small rodents. They are suitable for animals of 3–10 kg, and a minimum flow rate of 100–300 ml/kg/min is needed to prevent rebreathing.

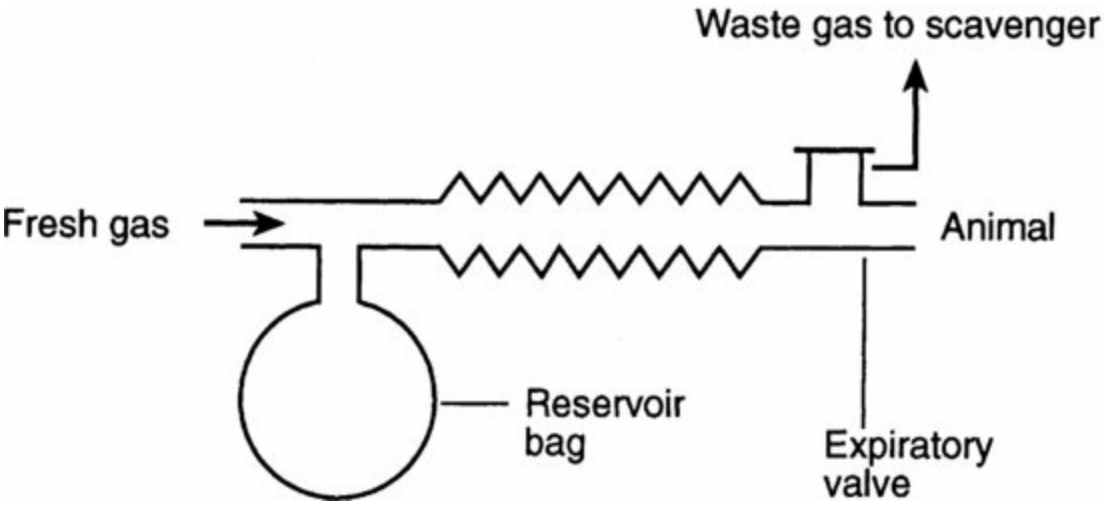
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FIGURE 9.3 Bain co-axial circuit.



The *Magill circuit* (Figure 9.4) is suitable for animals between 10 and 40 kg. A fresh gas flow of 80–90% of minute volume (respiratory rate×tidal volume) is needed, making this economic to use in these larger animals. This circuit is only suitable for animals of 10 kg and over because the valve causes high resistance and it has a relatively high dead space.

FIGURE 9.4 Magill circuit.

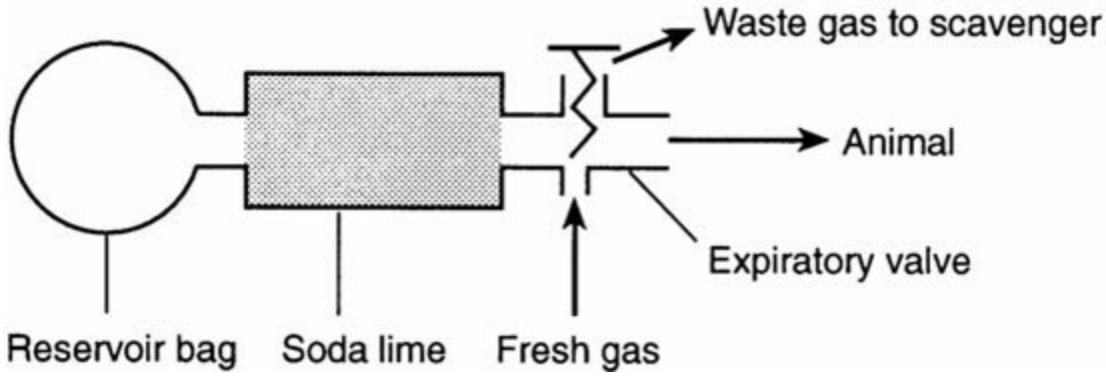


In rebreathing circuits, carbon dioxide in the exhaled gases is absorbed by soda lime so that the anaesthetic vapour can be re-circulated with the addition of fresh oxygen. The required gas flow rate is low, and re-use of expired anaesthetic vapour makes them very economical. Nitrous oxide should not be used with closed systems, since it is impossible to monitor the concentration in the recycled gas. One disadvantage is that the packed soda lime increases the resistance to respiration and dead space. This makes these systems unsuitable for animals under 15 kg in weight. The conservation of heat in the system may give rise to heat stroke in sheep. Sevoflurane should not be used with rebreathing systems.

In the *To and fro system* (Figure 9.5), the canister containing soda lime is placed between the animal and the reservoir bag to absorb the carbon dioxide. The dust from the soda lime may be inhaled and cause inflammation in the respiratory tract.

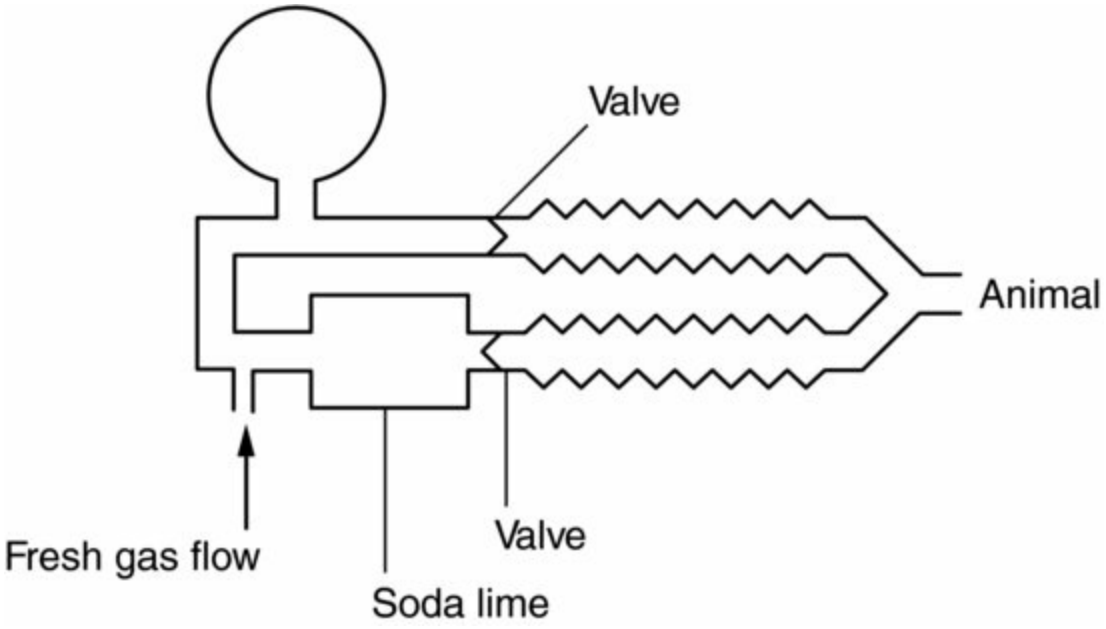
FIGURE 9.5 To and fro system.

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In *Circle systems* (Figure 9.6) there is a circular flow of gas controlled by two unidirectional valves. They are designed for large animals, as there is high resistance in the system.

FIGURE 9.6 Circle system.



Maintenance of anaesthesia: face masks and endotracheal tubes

The anaesthetic vapour and carrier gas are delivered from the circuit to the animal via a face mask or endotracheal tube. Face masks must fit snugly over the nose and mouth but not rub the eyes. Gas invariably leaks from around the mask, contributing to pollution of the atmosphere, so these should either be used in a flow hood, or a face mask incorporating a gas-scavenging system used. Face masks require a flow rate of three times the animal's minute volume.

Endotracheal tubes are rubber or plastic tubes inserted into the trachea. These are available in different sizes for different species, with or without an inflatable cuff, which is used to make an airtight seal in the trachea. The tube provides a patent airway so that artificial respiration may be given if required. Endotracheal intubation requires skill, and in some species the use of a laryngoscope. After induction of anaesthesia, the lubricated tube is passed over the tongue and epiglottis, and through the larynx. Once in situ, the cuff, if present, is inflated to seal the trachea and prevent the animal from breathing around the tube. Inflatable cuffs should not be used for tubes smaller than 5 mm diameter.

The size of tube used depends on the size of the animal, but as a rough guide adult beagles need a 9 or 10 mm tube with a cuff, cats need a 3–5 mm tube without a cuff and rats need a 16–12 G arterial cannula or modified urinary catheter. Dogs, cats and primates may be intubated while lying in left lateral recumbency (right-handed operator). An assistant holds the head and neck extended and lifts the upper jaw. The operator pulls the tongue forward and down, and usually the larynx can then be visualised. The tube is then passed gently through the glottis into the trachea. In cats, the larynx should be sprayed with local anaesthetic

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prior to passage of the tube to prevent laryngeal spasm.

Pigs have a particularly long larynx, and a laryngoscope is required to illuminate the larynx and hold down the tongue and epiglottis.

Small animals can be intubated via surgical implantation of a tube in the trachea in non-recovery procedures, or by passing a tube carefully into the mouth. For rats the animal is placed on its back and a laryngoscope or otoscope inserted into the mouth to visualise the larynx[14]. An alternative to a laryngoscope can be fashioned from a syringe barrel[15]. The tube can then be inserted through the glottis. For rabbits, the animal is placed in ventral recumbency and the head and neck extended by an assistant so the neck is straight. The tube is inserted in the mouth to one side of the incisors then straightened and advanced as far as the glottis. The tube is then advanced into the larynx during the inspiratory phase of respiration. Alternatively, an assistant stands in front of the prone rabbit holding its head up and pulling the tongue forward. The operator stands behind the rabbit, and places the tube as above[16,17,18].

Endotracheal intubation in mice, gerbils and hamsters is difficult and requires skill and purpose-made apparatus1. This often has to be done following induction with injectable anaesthetics, because the animal may wake from inhalation anaesthesia in the time taken to place the tube. A method has been described in which intubation is facilitated, allowing for intubation after inhalation induction[19].

An alternative to endotracheal intubation is the use of a nasal catheter. This is effective in many rodents as they are nasal breathers. Small nasogastric tubes, urinary catheters or intravenous catheters can be used. After induction of anaesthesia the catheter is inserted through the external nares and into the nasal passage, by applying gentle pressure and directing the catheter medially and ventrally. The typical gas flow rate required is three times the minute volume. Animals may sneeze if too lightly anaesthetised, and there may be mild epistaxis, which can be stopped by applying pressure to the side of the nose[20].

Health and safety

Volatile anaesthetic agents pose some risk to human health. There may be alterations in cardiorespiratory function, hepatotoxicity occurs infrequently but has been reported and all modern agents can trigger malignant hyperthermia[21]. Prolonged exposure to some agents can cause abortion. Each volatile agent has a defined occupational exposure limit (OEL), which should not be exceeded, and so either an active or passive scavenger system must be used to extract the waste gases and dispose of them safely. A length of tubing attached to the expiratory port of the circuit can be used to duct waste gases to the outside, or into activated charcoal. Alternatively, the apparatus may be used in an extraction cabinet. Activated charcoal canisters must be weighed regularly and changed when they reach a specified weight. Activated charcoal will not remove nitrous oxide.

Injectable anaesthesia

Injectable agents are simple to administer with a minimum amount of equipment. This is useful when operating in the field or when equipment for administration of volatile anaesthetic agents is not available. With intravenous injection, anaesthesia is induced immediately, minimising stress. They are particularly useful for the induction of anaesthesia, which can then be maintained by inhalation, or for short-duration anaesthesia.

Modern injectable agents are available that are short-acting, and which can be given by intravenous infusion, allowing for improved control of the depth of anaesthesia and overcoming some of the disadvantages of using injectable agents.

When injectable agents are used it is still sometimes helpful to administer oxygen, to prevent development of hypoxia, for example during long-term anaesthesia lasting for more than 1 h, in which respiratory depression can lead to hypercapnia and acidosis. The ability to administer oxygen and control ventilation helps control many potential difficulties encountered in injectable anaesthesia.

Induction

Injectable agents are given either intravenously directly into the bloodstream, or via another parenteral route from which they are absorbed into the blood. Intravenous injections usually act very rapidly, producing loss of consciousness almost immediately. The calculated dose can be administered slowly, allowing time for the drug to circulate before deciding whether more is required. The drug can, therefore, be administered to effect and titrated to the exact requirements of the animal. Intravenous administration can be facilitated by applying local anaesthetic to the site of venipuncture prior to injection.

The small size or problems of restraint in many laboratory animals means that injectable agents are most frequently given via the intramuscular or intraperitoneal routes as a single dose. With this route, a large total dose is needed, and absorption can be

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slow and unpredictable, leading to variable induction. Giving the drug as a single bolus can result in overdosage, which is difficult to reverse, and the animal must be weighed first to ensure accurate dosing. Residual drug effects persist for long periods and full recovery can be prolonged. There is also the possibility of accidental injection into a blood vessel or major abdominal organ, which could have serious consequences. Ideally injectable anaesthetics should have a wide safety margin, and be able to be given in a small volume through a small needle.

A considerable amount of skill is required to perform intravenous injection. For all other routes less skill is required, but it is not possible to dose to effect, since the response to injection is slow. For injectable agents, more than inhaled agents, the level of anaesthesia achieved depends on the level of stimulation of the animal at the time. If an animal is stressed, for example due to poor handling or restraint in the pre-anaesthetic period, it will require a higher dose of anaesthetic agent, leading to a greater risk of overdosage and adverse effects. An animal that is given an injection and left undisturbed may be apparently deeply anaesthetised, but if stimulated it may respond by reflex movements of the limbs and an increase in the depth and rate of breathing. This does not necessarily mean that a further injection of anaesthetic is required: if more is given, and stimulation then stops, there is a risk that the level of anaesthesia will become dangerously deep. The depth and duration of anaesthesia produced is therefore variable.

Maintenance

Injectable anaesthetics produce variable periods of anaesthesia depending on the agent used. If maintenance is required this can be achieved by giving intermittent top-up injections, by continuous intravenous infusion, or by using an inhaled agent for maintenance. The response to injectable anaesthetic agents varies with the individual animal, being affected by sex, strain and age. This is true for all anaesthetics, but adjusting the depth of injectable anaesthesia to account for these differences is more difficult than for inhalation anaesthesia.

Intravenous injections allow dosing to be adjusted according to the individual animal's response, so there is less likelihood of over- or under-dosing. Many drugs and combinations can be given by constant intravenous infusion for prolonged anaesthesia.

Giving intermittent injections for maintenance inevitably results in variable depth of anaesthesia during the procedure. Monitoring the depth of anaesthesia is therefore particularly important, so that supplemental injections can be given in good time to prevent the animal from feeling pain, as there will be a time lag between the top-up injection and deepening of anaesthesia. *It is not acceptable to give a further dose by injection as the animal starts to wake up.* It is important to be familiar with the techniques required for the administration of injectable agents, and the type of anaesthesia obtained with particular anaesthetics in the type of animal to be used.

Recovery

Most injectable anaesthetics are excreted by the kidneys after metabolism in the liver. This process can be prolonged, leading to slow recovery from anaesthesia, but may also affect hepatic enzymes, interfering with some studies.

The action of some injectable anaesthetic agents can be reversed using specific antagonist drugs. Several antagonists are available commercially, and these can partially reverse some anaesthetic combinations. They have several major benefits.

- By reversing the sedation and reducing sleep time, animals become active more rapidly, and are less at risk of hypothermia.
- Many anaesthetic drugs depress respiration, and this depression can be promptly and effectively reversed with antagonist drugs.
- An additional advantage of sequential analgesia can be obtained when reversing pure agonist opioids, such as fentanyl, with partial agonists such as buprenorphine. The side effects produced by fentanyl are reversed, but the analgesia persists because of the action of the bupenorphine1.

Injectable agents

Many injectable agents are available. The quality of anaesthesia produced by different agents varies considerably. Very few anaesthetic agents can produce general anaesthesia when used alone, and most injectable agents are given in combinations. Even with agents that can induce general anaesthesia alone, reduction of anxiety with suitable premedicants, additional analgesia or use of an inhaled agent for maintenance may be required. Table 9.3 gives doses of anaesthetic drugs for small laboratory animals.

Table 9.3 (a) Anaesthetic and other drug dose rates for in rodents and rabbits1, 20, 25,26. (b) Analgesic doses for small animals1, 20,25. (c) Drug doses for use in dogs and ferrets 27,28. (d) Anaesthetic and analgesic dose rates for chickens. (e) Doses for sedatives, anaesthetics and analgesics in ruminants. (f) Sedative, anaesthetic and analgesic drug dose rates for pigs. (g) Dose rates for anaesthetics and analgesics in primates.

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(a) Anaesthetic and other drug doses for use in rodents and rabbits[1,20,25,26]

Drug	Type of small animal: Guinea pig Dose	Guinea pig Route	Mouse Dose	Mouse Route	Rabbit Dose	Rabbit Route	Rat Dose	Rat Route	Hamster Dose	Hamster Route	Gerbil Dose	Gerbil Route	Comments	Duration of anaesthesia	Sleep time
Hynorm (ml/kg) (fentanyl/ fluanisone)	0.65	i.m.	0.5	i.p.	0.22	i.m.	03–0.6	i.m. or i.p.	0.5	i.m	–	–	Sedation only, good analgesia, poor relaxation. Reverse with naloxone 0.1 mg/kg.	–	–
Alphaxalone (mg/kg)	40	i.v.	10–15	i.v.	6–9	i.v.	10–15	i.v.	150	i.p.	–	–	Surgical anaesthesia only if i.v.	5 min	10 min
Hypnorm (ml/kg) + diazepam (mg/kg)	1+2.5	i.m. + i.p.	0.3+5	i.m. + i.p.	0.3+2.5	i.m. + i.v.	0.3+2.5	i.m.	1+5	i.m. or i.p.	0.3+5	i.p.	Good surgical anaesthesia. Do not mix in same syringe.	45–60 min	3–4 h†
Hypnorm (ml/kg) + midazolam (mg/kg)	0.5–1 + 2.5 (or 8*)	i.p.	10*	i.p.	0.3+2	i.m. + i.v.	0.6+2.5 (or 2.7*)	i.p.	0.05–1+5 (or 4*)	i.p.	0.5–1+5	i.p.	Good surgical anaesthesia. Prolong with additional Hypnorm at 0.1–0.3 ml/kg every 20–40 min. Reverse with naloxone 0.01–0.1 mg/kg i.v. or i.p. or buprenorphine 0.05 –0.1 mg/kg i.p. or s.c., or butorphanol 2.0 mg/kg i.p. or s.c	40–60 min	3–4 h†
Ketamine (mg/kg) + diazepam (mg/kg)	100+5	i.p.	150+5	i.p.	25+5	i.m.	75+5	i.p.	–	–	50+5	i.p.	Light anaesthesia	20 min	2 h

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Ketamine (mg/kg) + medetomidine (mg/kg)	40+0.25	i.p.	75+0.5–1	i.p.	15+0.25	s.c. or i.m.	60–75+0.5	i.p.	100+0.25	i.p.	75+0.5	i.p., s.c. or i.m.	Good surgical anaesthesia. Reverse with atipamezole 1 mg/kg s.c. or i.p. Can dilute 1:10 with sterile water for accurate dosing in small animals. Can be variable in guinea pigs.	20–30 min	2–4 h†
Ketamine (mg/kg) + xylazine (mg/kg)	40+5	i.m.	80–100+10	i.p.	25–35+3–5	i.m.	90+10	i.p.	50–200+5–10	i.p.	100+2	i.p.	Surgical anaesthesia, may be some respiratory depression	20–30 min	2–4 h†
Propofol (mg/kg)	10	i.v.	20–30	i.v.	5–10	i.v.	10	i.v.	10	i.v.	–	–	Surgical anaesthesia only if i.v. Total i.v. anaesthesia can be achieved in rabbits by infusion at 0.2–0.6 mg/kg/min.	5 min	10 min
Medetomidine (mg/kg)	–	–	0.03–0.1	s.c.	0.25	s.c.	0.03–0.1	s.c.	0.1	s.c.	0.1	s.c.	Sedation only. Reverse with atipamezole 1 mg/kg s.c. (0.2 mg/kg i.v. in rabbit)		
Fentanyl (mg/kg) + medetomidine (mg/kg)	0.16+0.4	i.p.	Do not use		0.08+0.33	i.v.	300+0.3	i.p.	–	–	–	–	Variable anaesthesia in guinea pigs. Reverse with atipamezole 1 mg/kg s.c. plus nalbuphine 1 mg/kg s.c. or butorphanol 2.0 mg/kg i.p. or s.c.	45–60 min	2–4 h
Doxapram (mg/kg)	5–15	i.v., i.m.	5–10	i.v.	2–5	i.v.	5–10	s.c, i.v. or i.p.	5–10	s.c.	5–10	s.c.	To reverse respiratory depression		
Atropine (mg/kg)	0.05	s.c.	0.05	s.c.	–	–	0.05	s.c.	0.04	s.c.	0.04	s.c.	To reduce salivary and bronchial secretions		

*Mixture of 1 part Hypnorm, 2 parts water for injection and 1 part midazolam. Dose in ml/kg.
†Recovery time reduced if specific antagonists are administered

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(b) Analgesic doses for small animals[1,20,25]

	Mouse			Rat			Guinea pig			Hamster			Gerbil			Rabbit		
Analgesic	Dose (mg/kg)	Route	Interval (hourly)	Dose (mg/kg)	Route	Interval (hourly)	Dose (mg/kg)	Route	Interval (hourly)	Dose (mg/kg)	Route	Interval (hourly)	Dose (mg/kg)	Route	Interval (hourly)	Dose (mg/kg)	Route	Interval (hourly)
Buprenorphine	0.05–0.1	s.c.	8–12	0.05–0.1	s.c.	8–12	0.05–1	s.c.	8–12	0.05	s.c.	8–12	0.1–0.2	s.c.	6–8	0.01–0.05	s.c., i.v.	6–12
Butorphanol	1–5	s.c.	4	0.5–2	s.c.	2–4	0.5–1	s.c.	24	0.4	s.c.	8–12	2	s.c. or i.p.	2–4	0.1–0.5	i.v.	4
Flunixin	2.5	s.c., i.m.	12	2.5	s.c., i.m.	12	2.5	i.m. or s.c.	24	2.5	s.c.	12	2.5	s.c	12–24	1.1	s.c., i.m.	12
Ibuprofen	30	Oral	4	15	Oral	14	10	i.m.	4	–	–	–	–	–	–	10	i.v.	4
Meloxicam	5	s.c.	24	1.0	s.c., oral	12–24	0.1–0.3	s.c., oral	24	–	–	–	–	–	–	0.2	s.c.	24
Morphine	2–5	s.c.	2–4	2–5	s.c.	24	10	s.c., i.m.	4	–	–	–	–	–	–	2–5	s.c., i.m.	2–4
Pethidine	10–20	s.c., i.m.	2–3	10–20	s.c., i.m.	2–3	10–20	s.c., i.m.	2–3	10–20	s.c.	2–3	10–20	s.c.	2–3	10	s.c., i.m.	2–3
Codeine	60–90 20	Oral, s.c.	4	60	s.c.	4	–	–	–	–	–	–	–	–	–	–	–	–
Nalbupine	4–8	s.c.	1	1–2	s.c.	3	–	–	–	–	–	–	–	–	–	1–2	i.m., i.v.	4
Carprofen	2–4 4	s.c., oral	24	2–5	s.c	24	–	–	–	–	–	–	5	s.c.	8–12	4 1.5	s.c., oral	12–24

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(c) Drug doses for use in dogs and ferrets[27,28]

Drug	Dose (dog) (mg/kg)	Dose (ferret) (mg/kg)	Notes
Sedatives/tranquillizers			
Medetomidine	0.010–0.080 i.m., s.c. or i.v.	0.1 i.v., i.m.or s.c.	Use alone for sedation, or for premedication. Duration and degree of sedation are dose-dependent. Peak effect after 10–15 min. Reverse with atipamezole (equal volume to medetomidine used). If used for premedication, give low dose and reverse with atipamezole after induction of anaesthesia. May induce vomiting.
Medetomidine + butorphanol	0.01–0.025 + 0.1 i.m. or i.v.		Can be mixed together and given in one syringe. Produces sedation and analgesia. Time to peak effect 20 min. Reverse with atipamezole (equal volume to medetomidine).
Xylazine	1–3 i.m., s.c.	1 i.v., i.m. or s.c.	Produces sedation with some analgesia. Causes hypotension, cardiovascular and respiratory depression. May induce vomiting. Can use for premedication.
Midazolam + ketamine	0.1–0.3 + 5.5 i.v. or i.m.	0.2 + 10 i.m	If given i.m. may produce variable sedation or light anaesthesia. Time to peak effect 2–10 min. Light anaesthesia of 20–30 min duration, with good maintenance of blood pressure.
General anaesthetics			
Medetomidine + ketamine	0.04 + 5–7.5 i.m.	0.05–0.1 + 4–8 i.m.	Time to peak effect 5–10 min. Produces anaesthesia lasting 30–50 min. Reverse medetomidine with atipamezole (equal volume to medetomidine). May be cardiovascular depression with peripheral cyanosis.
Alphaxalone	2–3 i.v.	8.2–11 i.m. or i.v.	For induction of general anaesthesia. Use lower dose in premedicated animals. Produces anaesthesia of short duration, which can be maintained with further doses of alphaxalone or by inhalation.
Propofol	1[a], 4[b] or 6.5[c] i.v.	10[c] i.v.	[a] If premedicated with an α_2-agonist [b] If premedicated (non-α_2-agonist) [c] If unpremedicated For induction of short duration anaesthesia (5 min) or prior to maintenance by intravenous infusion or by inhalation
Ketamine + diazepam	5 + 0.1–0.4 i.v.	25 + 2 i.m.	Surgical anaesthesia 30 min. Less respiratory depression than with α_2-agonists.
Ketamine + xylazine	15 + 1 i.m.	25 + 1–4 i.m.	Similar to medetomidine with ketamine. Administer xylazine first then ketamine. Time to effect 7–10 min. Anaesthesia lasts 20–25 min.

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(c) Drug doses for use in dogs and ferrets[27,28]

Drug	Dose (dog) (mg/kg)	Dose (ferret) (mg/kg)	Notes
Analgesics			
Meloxicam	Initial dose 0.2 s.c., i.v. or oral	0.2 s.c. or i.m. 0.3 p.o.	Once daily dosing. For relief of post-surgical pain. Reduce to 0.1 mg/kg for subsequent doses. Can also be used pre-surgery as part of a balanced anaesthetic regime.
Carprofen	4 i.v., s.c., oral	1 p.o. or s.c.	Can use pre surgery as part of balanced anaesthetic regime. For post-surgical analgesia administer once daily for up to 5 days.
Ketoprofen	2 s.c., i.m. or i.v.	2 s.c.	Once daily for up to 5 days
Butorphanol	0.2–0.4 i.v.	0.25 s.c. or i.m.	For relief of moderate pain of short duration (30–60 min)
Buprenorphine	0.01–0.02	0.05 s.c. or i.m.	Use pre surgery to provide sedation and analgesia, and for post-surgical analgesia. Lasts up to 12 h. Can repeat after 3–4 h if required.
Methadone	0.5–1 s.c., i.m. or i.v.		For analgesia, or can be used for premedication before induction with propofol and maintenance with propofol or isoflurane.
Other drugs			
Atropine sulphate	0.01–0.03 i.v.	0.05 s.c.	To control bradyarrhythmias
Glycopyrrolate	0.002–0.1 i.v., i.m.	0.01 s.c.	To control bradyarrhythmias. Can also use to reduce salivary and bronchial secretions.
Doxapram	5–10 i.v.	1–2 i.v.	To reduce respiratory depression. Can repeat after 20 min.

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(d) Anaesthetic and analgesic dose rates for chickens		
Drug	**Dose (mg/kg) and route**	**Notes**
Anaesthetics		
Propofol	10 i.v.	Best given intravenously, via the alar or jugular veins. Short-acting, 5–10 min of anaesthesia, with rapid recovery. Useful as for induction, followed by endotracheal intubation and maintainance with a volatile agent, such as isoflurane.
Alphaxalone	2–4 i.v.	Gives approximately 20 min of surgical anaesthesia. This can be extended by incremental dosing without significantly increasing the recovery time.
Ketamine+ midazolam or diazepam	20 i.m. +4 or 1.5	Produces light anaesthesia. Less reliable than propofol or alphaxalone.
Metomidate	10–20 i.m.	Sedation and immobilization for minor procedures.
Isoflurane	3–5% induction, then 1–3% maintenance. By inhalation	Anaesthetic of choice. High gas flow rates needed (770 ml/min for 2.5 kg bird). Changes in anaesthetic depth occur very rapidly. Oxygen should be provided after procedure to flush isoflurane out of air sacs
Analgesics		
Butorphanol	3–4 i.m.	Analgesic of choice
Buprenorphine	0.01–0.05 i.m.	
Meloxicam	0.5–1 i.m. or p.o.	

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(e) Doses for sedatives, anaesthetics and analgesics in ruminants			
Drug	Dose in sheep and goats	Dose in cattle*	Notes
Sedatives			
Diazepam	2 mg/kg i.m. or 1 mg/kg slowly i.v.		Good tranquillization in sheep
Midazolam	0.5 mg/kg i.v.		Similar tranquillization to diazepam
Acepromazine	0.05–0. 1 mg/kg i.m.		Good sedation, but no analgesia
Medetomidine	10 µg/kg i.m.		Deep sedation and some analgesia, with bradycardia and marked respiratory depression. Use with caution in sheep.
Xylazine	Use with extreme care; risk of death from respiratory failure.	0.05–0.3 mg/kg i.m.	Sedative of choice for cattle. Produces dose-dependent sedation. At the higher dose rates, animals become recumbent and may remain sedated for several hours. It may be possible to intubate cattle under deep xylazine sedation.
Anaesthetics			
Propofol	4–5 mg/kg i.v.	4–5 mg/kg i.v.	Injectable agent of choice. Produces 5 min surgical anaesthesia. Reduce dose by half following xylazine sedation. Sufficient to intubate for maintenance with inhalation. Incremental doses or an infusion can be given for maintenance, at 0.4–0.6 mg/kg/min.
Alphaxalone	2–3 mg/kg i.v.		Up to 15 min surgical anaesthesia. Incremental doses at 1–2 mg/kg i.v. every 15–20 min will produce stable long-term anaesthesia.
Ketamine + diazepam or midazolam	4 mg/kg i.v. +2 mg/kg i.v. or 1 mg/kg i.v.		Light anaesthesia
Isoflurane	1.5–3%	1.5–3%	For maintenance after induction with injectable agents.
Analgesics			
Buprenorphine	0.005–0.01 mg/kg i.m.		Lasts 4–6 h
Butorphanol	0.5 mg/kg s.c.	0.2–0.5 mg/kg s.c.	Lasts 2–3 h in sheep. Has been given to cattle at 24 h intervals.
Morphine	0.2–0.5 mg/kg i.m.		Total dose should not exceed 10 mg in sheep. Lasts 2–3 h.
Flunixin	2.2 mg/kg i.v. or i.m.	2.2 mg/kg i.v.	Administer once daily for 5 days
Carprofen	1.4 mg/kg s.c.or i.v.		Administer once daily
Ketoprofen	3 mg/kg i.v. or i.m.	3 mg/kg i.v. or i.m.	Administer once daily for up to 3 days
Meloxicam	0.5 mg/kg given s.c. or i.v.	0.5 mg/kg given s.c. or i.v.	Single dose, provides 72 h pain relief
Tolfenamic acid		4 mg/kg i.v.	Single dose, provides 72 h pain relief

*Non-steroidal anti-inflammatory drugs (NSAIDs) are the normal analgesics used in cattle. They may be safely given to animals previously sedated with xylazine, which itself has analgesic properties at the higher dose rate (0.2–0.3 mg/kg). There is little information on the effectiveness or suitability of opioid drugs in cattle.

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(f) Sedative, anaesthetic and analgesic drug dose rates for pigs		
Agent	Dose and route	Comments
Sedatives		
Azaperone	1–2 mg/kg deep i.m.	Sedative licensed for use in pigs. Use a 40 mm needle. Higher dose rate for nervous animals. Leave animal undisturbed for 20 min after injection.
Diazepam	2.0 mg/kg i.m.	
Acepromazine	0.1 mg/kg i.m.	
General anaesthesics		
Alphaxalone	2 mg/kg i.v. after azaperone sedation	Injectable anaesthetic agent of choice. Surgical anaesthesia for 10–15 min. Maintain with incremental doses or by inhalation.
Propofol	2.5–3.5 mg/kg i.v.	Short-duration anaesthesia. Can maintain with i.v. infusion at 8–9 mg/kg/h (4–8 mg/kg/h if premedicated). Can produce respiratory depression.
Xylazine + ketamine	1 mg/kg i.m. or i.v. + 2–5 mg/kg i.v.	Give xylazine, then follow with ketamine after 10 min.
Analgesics		
Buprenorphine	0.005–0.02 mg/kg i.m. or i.v.	Give 6–12 hourly
Morphine	0. 2 mg/kg i.m.	Give 4 hourly up to a maximum of 20 mg
Pethidine	2 mg/kg i.m.	Give 2–4 hourly up to a maximum of 1 g in large pigs
Meloxicam*	0.4 mg/kg i.m.	May be repeated once after 24 h
Ketoprofen*	3 mg/kg i.m.	Give daily for up to 3 days
Tolfenamic acid*	2 mg/kg i.m.	Give once only. Will last for 72 h
Flunixin*	1 mg/kg i.m.	Give daily for up to 3 days
Carprofen	1–2 mg/kg s.c.	Give daily for up to 3 days
Aspirin	10–20 mg/kg	Post-operatively, give 4 hourly

*Licensed product available for use in pigs.

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(g) Dose rates for anaesthetics and analgesics in primates

Drug	Dose and route	Comments
Sedatives and tranquillizers		
Ketamine	5–25 mg/kg i.m.	Drug of choice for tranquillization. Peak effect in 5–10 min, lasts 30–60 min
Medetomidine	50–100 µg/kg i.m.	Moderate sedation. Reverse with atipamezole 250–500 g/kg i.m.
Diazepam	1 mg/kg i.m.	Sedation, but insufficient alone for handling large primates. Can combine with ketamine
General anaesthetics		
Medetomidine + ketamine	50–100 µg/kg i.m. + 5–10 mg/kg im.	Surgical anaesthesia with good relaxation lasting 30–40 min. Reverse medetomidine with atipamezole
Diazepam + ketamine	1 mg/kg i.m. + 15 mg/kg	Light surgical anaesthesia lasting 30–40 min
Propofol	7–8 mg/kg i.v.	Administer slowly i.v. to effect after ketamine sedation. To be followed by endotracheal intubation and maintenance using isoflurane or sevoflurane. Can also maintain by propofol infusion at 0.5±0.15 mg/kg/min
Alphaxalone	10–12 mg/kg i.v. or 12–18 mg/kg i.m.	If given i.m. produces sedation in small primates. Can give i.v. to effect to produce general anaesthesia.
Analgesics		
Buprenorphine	0.01–0.03 mg/kg i.m. or i.v.	Analgesia lasts 6–8 h
Morphine	102 mg/kg s.c. or i.m.	Lasts 4 h
Meloxicam	0.1–0.2 mg/kg p.o.	Administer once daily for 3 days
Carprofen	3–4 mg/kg s.c.	Once daily for 3 days

i.m., intramuscular; i.p., intraperitoneal; i.v., intravenous; p.o., per os (by mouth); s.c., subcutaneous.

Propofol

This should be given by slow intravenous injection, as rapid administration may induce apnoea. It acts rapidly, inducing

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anaesthesia smoothly without excitatory side effects. It is ultra-short acting, and recovery is rapid and smooth. It can be used by continuous infusion for prolonged anaesthesia. It can be used safely in cats, dogs, monkeys, pigs and rabbits, and can be combined with a wide range of premedicants, analgesics and inhaled agents. Utility in small rodents is limited, however, due to the need for intravenous administration.

Alphaxalone

This is non-cumulative and produces good muscle relaxation with excellent cardiovascular stability and minimal respiratory depression except at high doses[22]. Intravenous administration rapidly results in anaesthesia, but the calculated dose should be given slowly, over 60 s, to avoid apnoea. It can be used for induction of anaesthesia prior to maintenance with inhalation agents, for short-duration anaesthesia or for long-term anaesthesia by intermittent injection or continuous infusion. Analgesia produced is limited, and additional analgesia may be required.

Recovery following anaesthesia is rapid, but it is preferable that animals are left undisturbed in the recovery period, otherwise paddling, minor muscle twitching or more violent movements may be seen.

At the time of writing, there has been limited use of alphaxalone in laboratory animals. However, it has been shown to produce dose-dependent pre-emptive analgesia in rats[23], and has been used for anaesthesia in rats in studies requiring avoidance of significant respiratory and haemodynamic depression while facilitating mechanical ventilation[24]. It is likely that it will have a place in laboratory animal anaesthesia.

Pentobarbitone

This is a barbiturate that depresses the CNS and produces marked cardiovascular and respiratory depression. It has poor analgesic properties and very large doses are needed to reduce pain perception. It has a low therapeutic index: the lethal dose is only slightly above the clinical dose. Even after intravenous injection onset of anaesthesia is slow. Injections must be given very slowly to allow anaesthesia to deepen to its full extent before giving extra doses. If it is given intraperitoneally as a single bolus it has a poor safety margin, and mortality can be high. Recovery from anaesthesia is slow, and may be associated with convulsions and paddling. Its use is not recommended for recovery anaesthesia. In concentrated form, pentobarbitone is used for euthanasia.

Combination anaesthetics

Ketamine

This is an *N*-methyl-D-aspartate (NDMA)-receptor antagonist, which can be given by intramuscular or intravenous injection. It circulates rapidly after administration and produces analgesia but little muscle relaxation, and animals may exhibit tremors and remain responsive to external stimuli, particularly sound. Animals seem not to be aware of their surroundings, and analgesia varies with the species. It is the agent of choice for sedation of Old World monkeys. The corneal reflex is lost very quickly, and it is essential to protect the eyes with a bland ophthalmic ointment. The laryngeal and pharyngeal reflexes are maintained, but there is an increase in salivary secretions, which can cause airway obstruction, so ketamine is sometimes used with a drying agent such as atropine. There is good maintenance of blood pressure, but in most species the dose required to produce surgical anaesthesia causes severe respiratory depression and it is not used alone. It can be combined with xylazine, medetomidine, diazepam, midazolam or alphaxalone to produce general anaesthesia in many species (sheep, primates, pigs, rabbits and rodents). Using ketamine in combination with other agents also avoids side effects, such as muscle tremors.

Narcotic analgesics (opioid analgesics)

Opioids such as fentanyl, alfentanil and sufentanil are useful as premedicants, components of balanced anaesthetic regimes and in neuroleptanalgesia (see below). They are potent analgesics but have significant side effects such as respiratory depression. Fentanyl is a potent analgesic that lasts for 15–20 min. In rats, dogs and primates it has sedative effects, but in mice, cats and horses it causes excitement. Alfentanil is less potent than fentanyl but the onset of activity is more rapid. Sufentanil is 10 times more potent than fentanyl, but has not been used extensively in laboratory animals.

Neuroleptanalgesia

Combinations of opioid analgesics and sedatives produce neuroleptanalgesia, which is similar to a light plane of anaesthesia. The

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animal no longer responds to stimuli, but is not totally unconscious. Neuroleptanalgesics produce some respiratory depression and poor muscle relaxation, but when combined with benzodiazepines these problems are markedly reduced and a state of neuroleptanaesthesia may be produced, under which surgical procedures may be carried out. Combinations of fentanyl/fluanisone with either midazolam or diazepam can provide good anaesthesia for rodents and rabbits. Midazolam is water-soluble and can be administered in the same syringe as the fentanyl/fluanisone. It can also be diluted with sterile water for accurate dosing in small rodents.

To speed recovery and counteract side effects, the opioid component of neuroleptanalgesics can be reversed at the end of surgical procedures by either narcotic antagonists (such as naloxone) or partial agonists (such as buprenorphine or butorphanol), which also provide post-operative analgesia. Naloxone reverses analgesia as well as any side effects, so it must not be used unless surgery has been completed and adequate analgesia provided by some other means. Reversal is not always required, as most neuroleptanalgesics will wear off by themselves, but recovery without the use of antagonists can be prolonged.

Medetomidine and atipamezole

Medetomidine can be given by intravenous, intramuscular or subcutaneous injection. Animals should be left undisturbed for 10–15 min after administration for the drug to reach maximum effect, preferably in a quiet environment, as they may still react to noise. Medetomidine does not induce general anaesthesia when used alone, but is excellent when used in combinations. If used as a premedicant it reduces the requirement for inhaled anaesthetics markedly, and can be used with fentanyl/fluanisone to produce good anaesthesia and analgesia. It may also be used successfully with ketamine in many species. The biggest advantage of medetomidine is that it can be reversed very rapidly with the specific antagonist atipamezole. See also the section on premedication, above.

Long-term and non-recovery anaesthesia

Long-term anaesthesia may be required for some procedures. This can be achieved with intermittent top-up injections by the intramuscular or intraperitoneal routes, although the depth of anaesthesia can vary markedly. If the top up is not administered in time the animal may experience pain, so good anaesthetic monitoring is essential. Alternatively an anaesthetic can be given by continuous intravenous infusion (e.g. alphaxalone, propofol or fentanyl/midazolam). Short-acting agents enable the plasma concentration and depth of anaesthesia to be adjusted quickly, and lead to a rapid recovery. Many different types of infusion pump are available to facilitate administration. Long-term anaesthesia can also be achieved by the use of inhaled agents for maintenance, after induction with inhaled or injectable agents.

Many experimental protocols require terminal anaesthesia. There is little difference between recovery and non-recovery anaesthesia in terms of animal care and management, although some more invasive surgical techniques such as surgical exposure of a blood vessel for catheterisation and tracheostomy creation are more often used if the animal is not to be allowed to recover from anaesthesia.

Some anaesthetic agents are only suitable for non-recovery experiments, e.g. alpha-chloralose results in a very prolonged recovery period associated with considerable involuntary excitement, and urethane can produce severe peritoneal irritation and is carcinogenic. Chloral hydrate is a very poor analgesic in many species, and can cause paralytic ileus in the rat.

Recovery and post-anaesthetic care

At the conclusion of a procedure it is important that the animal wakes up as soon as possible, so that its physiology returns to normal. It is better to have a rapid recovery and provide adequate post-operative analgesia than to have prolonged anaesthesia. With inhaled agents the vaporizer can simply be switched off and the animal allowed to breathe oxygen for a few minutes until the anaesthetic gas has been exhaled, resulting in recovery within a few minutes. Injectable agents usually have to be metabolised by the liver and excreted by the kidneys. This process can be prolonged, and recovery from injectable anaesthetics usually takes longer than for inhaled agents. This increases the risk of hypothermia or dehydration. Some injectable drugs have specific reversal agents, the administration of which will lead to a return to consciousness within minutes. Reversal agents can also be used to reverse any respiratory depression, which develops in the post-operative period. Some injectable agents are ‘ultra-short-acting’, and are metabolised very quickly. Use of these newer drugs has overcome some of the problems previously associated with injectable agents.

The quality of care following recovery from an anesthetic can have a dramatic effect on the speed of recovery. If not cared for properly, semi-conscious animals may lie in their urine or faeces and develop skin lesions, or get bedding material in their eyes and

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noses. Their cage mates may attack them, and they may remain inappetant for long periods. Many of the monitoring procedures described below should be continued in the immediate recovery period. This helps to ensure that optimal attention is given to the animal, and that all the measures needed to ensure its comfort and well-being are undertaken promptly and effectively.

Since all animals will require some degree of special attention in the recovery period, it is preferable to provide a separate recovery area. This not only enables more appropriate environmental conditions to be maintained but also encourages individual attention and special nursing. Individual housing will often be indicated anyway to prevent problems such as injury of semi-conscious animals by their cage mates.

The recovery area should be stocked with a range of emergency drugs and equipment. Additional apparatus such as monitoring equipment may be needed after major surgery and/or prolonged anaesthesia.

The prevention and management of pain following a surgical procedure is a particularly important aspect of care in the recovery period. Pain can produce physiological changes that may alter the rate of recovery from surgical procedures, and that these changes may also affect the experimental results. Acute pain can be alleviated by the use of centrally or peripherally acting analgesic drugs administered systemically, by local anaesthetics or by the application of supporting bandages or other means of protecting and immobilising damaged tissues. The selection of a particular treatment will vary, depending upon the nature of the pain, its cause and its estimated severity and duration. For mild to moderate pain, non-steroidal anti-inflammatory drugs (NSAIDs) such as meloxicam, carprofen or flunixin may be sufficient. For moderate to severe pain, opioid analgesics such as buprenorphine may be needed.

The degree of personal attention given to animals will depend upon the species. Some animals may respond well to personal contact, whereas this may be stressful for others. Recovering animals should be left in a warm environment with subdued lighting, and be disturbed as little as possible. The animal should be on comfortable bedding and kept clean and dry. Animals may need additional fluid, given by injection or orally, and it may be appropriate to offer palatable food. Urine and faecal output should be monitored. All clinical observations and drugs administered must be recorded.

Local anaesthetics

Local anaesthetics act directly on nerves to block the conduction of impulses, thus eliminating pain and other sensations. Both local and regional anaesthesia are widely used in the management of pain in humans, and there are also well-established techniques in clinical veterinary practice. They are still relatively under-used in the smaller species of laboratory animals, principally because although pain sensation may be abolished there still remains the problem of restraint in these species.

Local anaesthetics may be applied topically, by injection, locally into the skin and/or underlying tissues in a specific area, or as a nerve block, for example epidural or paravertebral block, which results in desensitisation of a large area, known as regional anaesthesia. Local anaesthetics have an important role in providing anaesthesia and pain relief for large animal species. When combined with a sedative or hypnotic to provide humane restraint they can be used for major surgery and remove any of the risks associated with general anaesthesia.

Topical application

Some agents are available for direct administration to tissues. Drops can be used to desensitise the cornea and conjunctiva. An aerosol may be used for the larynx prior to intubation to prevent laryngospasm. For the skin, cream (e.g. EMLA, AstraZeneca) can be used to facilitate venipuncture.

Infiltration of the skin and underlying tissues

This method is useful for minor techniques, such as skin biopsies or suturing minor wounds. Injectable local anaesthetic is administered using a very fine needle. Agents such as lignocaine or bupivacaine can be infiltrated around the surgical site to provide analgesia during surgery or post-operatively. This method can be very useful for provision of post-operative analgesia, since there are no systemic side effects. Lignocaine preparations typically last 1–2 h, and bupivacaine can provide analgesia for up to 8 h20. Some preparations of lignocaine contain adrenaline to prolong efficacy: care should be taken not to overdose with these as this may delay wound healing.

Regional anaesthesia

Injectable local anaesthetic can be injected around a major nerve to desensitise a particular area. The use of nerve blocks for

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regional anaesthesia requires a detailed knowledge of anatomy and specific training in the techniques. The technique can be used for specific nerve blocks, such as the pudendal nerve for work in the pudendal region, and the cornual nerve block for dehorning or foot blocks using specific nerves to regions of the feet in horses. Epidural or spinal blocks involve injection of local anaesthetic into the epidural space or directly into the spinal cord to produce desensitisation of the region posterior to the site. This is a useful technique to provide anaesthesia of the pelvic region in cattle and sheep, and may be used post-operatively to provide continuing analgesia. A paravertebral block involves injecting local anesthetic around several lumbar spinal nerves as they emerge from the vertebral column to produce desensitisation of the flank. This method is useful in ruminants.

Although local anaesthetics may be very successfully used in the post-operative period as an adjunct to other methods of pain control, to provide adequate restraint and to reduce stress in most laboratory animals it is usually preferable to use general anaesthetics.

For doses of anaesthetic agents for each species, see Table 9.3.

Anaesthetic Management

All anaesthetic drugs will, if given in sufficient quantity, cause death. On the other hand, if an insufficient amount is given, the animal will feel pain. To maintain the animal between these two extremes, care must be taken in the maintenance of *physiological stability*, particularly *airway patency, respiratory function*, *circulatory function* and *body temperature*, and in monitoring the *depth of anaesthesia*, to ensure that the parameters of unconsciousness, analgesia and muscle relaxation are being met. Poor animal care during anaesthesia can result in prolonged recovery times, or in extreme cases, even death. The animal should be monitored frequently, at least every 5 min, or more often if stability has not been established, to ensure the animal is physiologically stable, and to determine the depth of anaesthesia. Records should be kept of the measurements to enable adverse trends to be spotted easily and corrective action taken. It also allows a series of anaesthetics to be compared. Anaesthetic monitoring depends on watching the animal closely and recording the measurements. An example of an anaesthetic record chart is shown at Figure 9.7.

FIGURE 9.7 Record of general anaesthetic.

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PIL holder ..... PPL No ..... Date .....

Species/strain ..... Age ..... Sex .....

Procedure .....

Pre-Op details

Temp ..... °C Pulse ...../min Resp ...../min

Eating ..... Behaviour ..... (0 = normal; 4 = grossly abnormal)

Weight ..... Risk ..... (0 = low; 4 = high)

Time																					
300																					
280																					
260																					
240																					
220																					
200																					
180																					
160																					
140																					
120																					
100																					
80																					
60																					
40																					
20																					

● Pulse ○ Respiration x Blood pressure

Drugs given																					Total dose
1																					
2																					
3																					

Recovery .....

Post operative care instructions .....

Personnel carrying out prolonged procedures may become fatigued, and steps should be taken to ensure that this does not impact on the care of the animal. There should be sufficient staff available to ensure that monitoring animals under anaesthesia is carried out effectively and diligently.

Physiological stability

There must be maintenance of adequate tissue oxygenation and removal of waste carbon dioxide during anaesthesia. The amount of oxygen reaching the tissues depends on several factors, as demonstrated by the oxygen flux equation:

$$O_2 \text{ available} = Q \times \text{arterial saturation}(\%) \times Hb \times K$$

where Q is cardiac output (depends on the efficacy of the heart) in millilitres per minute, arterial saturation (of the blood) depends on efficacy of the lungs, Hb is the haemoglobin level (g/ml) and K is a constant equal to 1.36.

Minor changes to one part of the equation may have little effect, but if there are changes in several parts the overall effect is magnified. Health problems can affect any or all parts of the equation, leading to a reduction in the quantity of oxygen available to the tissues. As general anaesthesia will reduce both cardiac output and lung efficiency, the combined effects of disease and anaesthesia can cause a severe reduction in tissue oxygen levels leading to hypoxia or even death. Animals must be monitored to ensure that there is adequate maintenance of tissue oxygenation. If anaesthesia is too deep the animal may be in danger of dying from respiratory and cardiovascular failure. The animal may exhibit slow shallow breathing, or deep gasping breaths, blue coloration of the mucous membranes and a fall in blood pressure. There may be a fixed, staring eye. Monitoring of the vital signs will detect these changes early on, and enable action to be taken to prevent death.

When monitoring animals under anaesthesia, it helps to remember A for **A**irway, B for **B**reathing and C for **C**irculation. The

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airway should be checked to ensure the animal can breathe and that the trachea is not blocked. For inhalation anaesthesia this should include checking that the anaesthetic machine is set up properly and that there is sufficient oxygen and anaesthetic vapour.

Breathing

Breathing is easily monitored, and assessments should be made of the *rate*, *depth* and *pattern* of breathing. This is done by watching the movement of the chest wall, or reservoir bag, or in larger animals by the use of an oesophageal stethoscope. An apnoea alarm can be useful. The effectiveness of pulmonary gas exchange can be assessed by the *colour of the mucous membranes*, and of blood being shed at the site of surgery. Virtually all anaesthetics cause some respiratory depression, leading to hypoxia and hypercapnia. Hypoxia may be indicated if the colour of blood or mucous membranes changes, but hypercapnia is not. Providing the animal with oxygen intra-operatively helps to prevent the development of hypoxia. Carbon dioxide concentration can be assessed by measuring the end-tidal CO_2, or by direct arterial blood-gas analysis. Transcutaneous O_2- and CO_2-monitoring equipment can be used in some species.

The respiratory rate usually falls during anaesthesia, and a fall of up to 50% from the normal is acceptable. If the rate continues to drop, there may be a problem. If the animal is not breathing the anaesthesia may be too light and the animal holding its breath, or conversely the anaesthesia may be too deep. This demonstrates the need to monitor more than one variable. Apnoea on induction of anaesthetic is common, particularly with the use of certain anaesthetic agents. If there is genuine apnoea, first ensure that the airway is clear: the pharynx or trachea may be blocked with mucus or blood, particularly in small animals, or the endotracheal tube may be kinked. Make sure that the thoracic movements are not restricted by the position of the animal, or by limbs being tied down too tightly. Check that undue pressure is not being placed on the chest wall from instruments and take care not to lean on the animal.

Respiration can be stimulated by moving the endotracheal tube, artificial ventilation, needle stimulation of the nasal philtrum or by respiratory stimulants (such as doxapram). For some anaesthetic combinations antagonists are available and these can be given if surgery has finished. It is advantageous if the animal has been intubated, or in non-recovery cases a tracheotomy tube has been placed, to enable a clear airway to be maintained, or to facilitate intermittent positive pressure ventilation (IPPV).

Circulation

Some anaesthetics cause a fall in cardiac output, and hypotension. Hypoxia and hypercapnia, resulting from respiratory depression, cause cardiac dysrhythmias. Loss of blood or body fluids can cause hypovolaemic shock and cardiac arrest. Circulatory function should be assessed frequently during anaesthesia. The simplest method of monitoring is to assess the rate, rhythm and quality of the *pulse*, using a superficial artery depending on the species. In larger species a stethoscope or oesophageal stethoscope can be used to indicate heart rate and rhythm. An electrocardiogram (ECG) gives an indication of cardiac function, but it only indicates the electrical activity of the heart. It is possible to have a normal ECG with a cardiac output of zero for a short while. A pulse oximeter indicates the blood oxygenation level and the pulse rate. Capillary refill time indicates tissue perfusion. This can be tested by applying pressure briefly to a mucous membrane to blanch it, then measuring how long it takes for the pink colour to return. It should take less than 2–3 s. Cardiac failure may occur suddenly, but more usually it is gradual in onset, and frequent monitoring can spot declining cardiovascular function allowing intervention to prevent a disaster.

It may be necessary to give warmed isotonic fluids such as saline or Hartmann's solution to support the circulation. Blood can be given in larger animals: transfusion reactions are rarely encountered on a first transfusion and never if another animal of the same inbred strain is used. A secure venous line should be established for infusion of fluids. This allows easy administration of analgesics and anaesthetics, and facilitates dosing with emergency drugs. If intravenous access is not available, fluids can be given subcutaneously.

Body temperature

It is essential to monitor this, particularly in small animals. All anaesthetics affect thermoregulation, and body temperature will fall unless measures are taken to prevent this. This is exacerbated by the flow of cold air from the anaesthetic machine, shaving the animal, use of cold skin preps, placing on a cold operating table, exposing viscera during surgery and administering cold fluids. Smaller animals have a larger surface area to volume ratio, so are particularly susceptible to heat loss. Animals less than 1 kg in weight will require supplementary heating to prevent a drop in body temperature.

Temperature can be monitored by feeling the animals' paws and ears, and by using a rectal or oesophageal probe, and core temperature can be compared with skin surface temperature. Ideally the difference should be less than 2–3°C. To reduce heat loss,

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any shaving should be over a minimal area, the fur should not be wetted excessively and dry drapes should be used. The environment should be warm, and ideally the operating table should be heated. Fluids to be administered should first be heated to 38°C. Heat loss can also be minimised by insulation with cotton wool, bedding, foil or bubble wrap. Additional heating can be provided with heat lamps, heating blankets, instant heat pouches or hot water bottles. Thermostatically controlled heating devices are available. A temporary heat source can be made simply by filling a glove with warm water. Care must be taken not to burn the animal.

Measures to prevent hypothermia must be continued throughout the recovery period. This can be achieved in small animals by using incubators. For adults, the temperature should be 25–30°C, and for neonates 35–37°C. Bedding also helps provide insulation. Sawdust, however, should not be used as this will stick to the animal's nose and mouth, or to wound surfaces. Hypothermia is the commonest cause of mortality in small rodents and slows recovery in larger species, so monitoring of body temperature and taking steps to prevent hypothermia are vitally important.

Other considerations

Care must be taken that the cornea does not dry out during anaesthesia, as the animal will not blink while asleep, and the flow of gases from the anaesthetic machine may exacerbate corneal drying. A bland ophthalmic ointment should be applied to protect the corneas and prevent drying.

Animals should be placed on a warm and comfortable surface during anaesthesia. For large animals in particular it is important to pad bony areas to prevent the development of pressure sores during long procedures. In some animals it may be necessary to empty the bladder during long procedures, and in any case the animal may pass urine so should be placed on absorbent material.

Depth of anaesthesia

Historically, anaesthesia in humans has been described as having four stages, with stage three (surgical anaesthesia) being divided into three planes. This classification was devised for use with a single volatile anaesthetic, and relies on assessment of cardiovascular and respiratory signs. In animals, with different species and combinations of drugs, this becomes unworkable. There exists a spectrum of levels of consciousness/depth of anaesthesia and it is essential to know approximately where in that spectrum the animal is at any time.

Anaesthesia aims to produce narcosis, relaxation and analgesia. Consciousness must be lost to a sufficient degree to prevent distress. Muscle relaxation can be assessed simply by evaluating muscle tone in the limbs or tail in smaller animals, or lower jaw in larger animals.

There must be sufficient analgesia to prevent the animal perceiving pain. This can be judged by the presence or absence of certain reflexes. The *pedal withdrawal reflex* is the most commonly used reflex. This involves extending one hind limb and pinching the toe-web (or toe base in small rodents), and assessing whether there is any withdrawal of the limb or evidence of conscious response. The tail or ear (e.g. in rabbits) may also be pinched. These responses are abolished if surgical anaesthesia has been reached.

Ocular reflexes can be useful in some circumstances but are variable indicators of the depth of anaesthesia. The palpebral reflex is lost at variable times depending on the species. In rodents it is hard to assess, and in rabbits it may not be lost until anaesthesia is very deep1. If ketamine is used the reflex is abolished very early. The corneal reflex is lost later and may be seen when drops are applied to moisturise the cornea. Observation of the position of the eyeball, the degree of pupillary dilatation, and whether nystagmus is occurring are all useful indicators once experience is gained with one species and a particular anaesthetic regime.

Since anaesthetics cause dose-dependent depression of cardiovascular and respiratory systems, changes in respiratory and heart rates, blood pressure can be used in conjunction with assessment of reflexes to determine the depth of anaesthesia. However, the way this depression manifests varies with different anaesthetics and it is difficult to generalise about the effects. It is usual for there to be a slight increase in heart and respiratory rates under anaesthesia in response to surgical stimulation, but there should be no pedal withdrawal reflex.

Equipment monitoring

Aside from monitoring vital signs, it is essential to monitor the *function of any equipment used*. Anaesthesia machines and infusion pumps may be fitted with alarms, which sound when the machines fail, or are empty.

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Anaesthetic Emergencies

Monitoring the parameters discussed above should allow for early detection of impending problems and appropriate corrective action before the development of an emergency. If an animal goes into circulatory arrest, and the cerebral circulation is not restored within 3 min, irreversible brain damage will occur. In a research setting, an animal which has experienced anaesthetic problems may no longer be a suitable experimental subject, and it may be appropriate to humanely kill the animal in such circumstances. If it is appropriate to try to resuscitate an experimental animal following an anaesthetic emergency, steps must be taken to maintain tissue oxygen levels, by ensuring the blood contains sufficient oxygen and is circulating properly.

Monitoring and resuscitation attempts are directed towards:

- A for airway,
- B for breathing,
- C for circulation.

First, check the airway is patent and that oxygen is available. Ensure the head and neck are extended and there is no blockage from mucus or blood. Then ensure the animal is breathing. In rodents and rabbits, a fall in respiratory rate to less than 40% of pre-anaesthetic value indicates impending respiratory failure1. If a volatile anaesthetic is used, reduce the inhaled concentration, and stop infusions of injectable agents or administer antagonists if available. Implement artificial ventilation if there is an endotracheal tube in place. If not, begin intermittent gentle compressions of the chest wall. It may be possible to stimulate breathing by stimulating the philtrum, or by use of respiratory stimulant drugs such as doxapram. Administer oxygen either directly via an anaesthetic machine or by placing the animal in an enriched oxygen atmosphere.

If there is evidence of circulatory failure, administration of fluids by the intravenous route may be sufficient to restore blood pressure. External cardiac massage can be achieved by placing the animal on its (right) side and compressing the chest wall firmly just behind the left elbow.

Hypothermia should be considered as a possible cause if recovery from anaesthetic is slow, particularly in small animals. Supplementary heating should be given as outlined above to raise the animal's core body temperature. Shivering to increase heat production results in an increase in oxygen demand; therefore, oxygen should be given to the hypothermic animal.

Most of the means for correcting cardiac failure used in humans such as defibrillation and drug treatments can be used in animals, but these are specialist techniques. If surgery is to be undertaken where there is a high risk of the development of an anaesthetic emergency then expert advice should be sought.

Muscle Relaxation During Anaesthesia

Relaxation is part of the triad of anaesthesia (narcosis, analgesia and relaxation). Muscle relaxation facilitates access to body cavities and to allow limbs to be moved easily. This reduces any damage caused to the muscles by using excess traction when trying to overcome muscle tone.

The abolition of muscle tone can be brought about in three ways, as follows.

1. Anaesthetic agents acting on the CNS can be used to decrease activity in ventral horn cells of the spinal cord. The degree of relaxation varies depending on the individual agent or combination used.

2. Local analgesics can be injected into a muscle mass or around nerves to isolate the muscle from nervous input and produce relaxation.

3. Neuromuscular blocking agents can be administered.

Neuromuscular blockers have no narcotic or analgesic effects. Their administration abolishes all ability to respond to pain but does not abolish the ability to feel pain. It is essential to ensure complete unconsciousness of all animals to which they are administered. The use of neuromuscular blocking agents is strictly controlled in many countries. In the UK, specific permission must be obtained from the Secretary of State at the Home Office and it must be clearly written into the Project Licence. Guidelines on the use of neuromuscular blocking agents are available on the Home Office website.

Monitoring anaesthesia under neuromuscular blocking agents

Many of the normal reflexes used to judge the depth of anaesthesia are abolished with neuromuscular blockers (e.g. palpebral reflex, pedal-withdrawal reflex) so other methods need to be employed to monitor the depth of anaesthesia under these

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conditions. Before the procedure to be carried out under neuromuscular block is performed it is essential to be familiar with the anesthetic regime to be employed to make sure that it provides a sufficient depth of anaesthesia for the procedure to be carried out. Then, under neuromuscular block, the following parameters can be used to monitor depth of anaesthesia:

- blood pressure,
- heart rate,
- twitching of muscles in response to surgical stimulation: this indicates that the depth of anaesthesia or neuromuscular block is inadequate,
- electroencephalogram (EEG): however, the interpretation of the EEG is often open to question and there is not always direct correlation with the level of narcosis. The reliability of this method is currently a matter of some discussion.

It is essential to use an endotracheal tube when using neuromuscular blockers to maintain the airway. An endotracheal tube facilitates IPPV and prevents air from being pushed into the stomach and intestines during forced breathing.

To measure the degree of neuromuscular blockade, a peripheral nerve stimulator can be used to determine muscle responses (the so-called train of four response). Alternatively the presence or absence of respiratory efforts can be evaluated. The intercostal muscles and diaphragm are generally the last muscles to become paralysed by neuromuscular blockers and the first to recover. However, monitoring these alone is inadequate to monitor the depth of anaesthesia.

Neuromuscular blocking agents

Sometimes simply called muscle relaxants, these produce paralysis of the skeletal muscles by blocking transmission of impulses from nerve endings across the neuromuscular junction (NMJ). There are two main types of neuromuscular blocking agent: depolarising agents/blockers and non-depolarising agents/blockers.

Depolarising agents

These act by mimicking the action of the normal transmitter (acetylcholine, ACh) at the neuromuscular junction. As a result these agents cause initial generalised muscle twitches, followed by complete skeletal paralysis. The main example is suxamethonium. Depolarising blockers are usually short-acting and are allowed to wear off naturally.

Non-depolarising agents

These are sometimes called competitive blockers since they work by competing with ACh for its receptors at the neuromuscular junction, reducing the degree of depolarisation of the membrane in response to nerve impulses. Examples include pancuronium, alcuronium, atracurium, vecuronium and gallamine. Their effects can be reversed by the administration of anticholinesterase drugs, which block the activity of enzymes that break ACh down, for example neostigmine, edrophonium or pyridostigmine.

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Chapter 10

Introduction to Surgery and Surgical Techniques

Surgery encompasses operative procedures involving cutting, abrading or otherwise physically changing body tissues and organs. Surgery therefore potentially constitutes a significant insult, and can cause many adverse effects, including pain, infection, blood loss, tissue damage and poor healing. This is true for both surgery carried out for clinical reasons and experimental surgery.

The main aim of any surgical procedure is that it is carried out skilfully with the minimum of risk and disturbance to the animal and without infection[1]. It is important to develop a high degree of skill and use rigorous aseptic technique to eliminate infection as a source of potential research variability. Aseptic technique is essential when undertaking both recovery surgery, on any species, and lengthy procedures carried out under terminal anaesthesia.

Surgery is a major insult with potentially devastating consequences. Good practice is *essential* if surgery is to be successful, and it is important to remember that luck is not a factor: *good techniques produce good results*.

Pre-Surgical Preparation

Before undertaking surgical procedures on laboratory animals careful planning, and a good appreciation of the anatomy and physiology of the appropriate area, are needed. Before starting it is important to prepare the animal, the instruments, the equipment, the facilities and the personnel. This preparation should begin well in advance of the procedure.

Animal

An appropriate animal should be chosen for the procedure, since a number of animal-related factors can affect healing (see Factors affecting healing, below), and it is essential to ensure the animal is fit for the procedure. Animals should be acclimatised to the environment for several days or weeks prior to the procedure, and many animals can be trained to accept the handling required for some procedures, reducing stress. Animals should be weighed, remembering that growing animals may need to be weighed regularly to ensure that the animal's correct weight is used for drug dose calculations etc.

Instruments and equipment

Ensure that appropriate equipment, instruments and consumables (e.g. gloves, swabs and drapes) are available and sterile. Choose appropriate instruments and equipment for the procedure, and prepare them appropriately in advance of the procedure. Other equipment such as an anaesthetic machine, monitoring equipment, operating light, adjustable table, heat pads and post-surgical care equipment may also be needed. A good, shadowless light source is essential, and for small-scale operations an operating microscope or a binocular loupe may be helpful.

The operating table itself should be very steady to permit precise movements by the operator. Facilities to adjust the height and tilt are useful to allow precise positioning of the animal and a comfortable, easily adjusted seat for the researcher may reduce fatigue and strain during long procedures.

For surgery on large animals there must be adequate equipment for lifting and moving an anaesthetised or recumbent animal, without injury to it or to the handler.

Facilities

Good surgery requires precision and clarity of thought, which is facilitated in a well-designed and appropriately stocked procedure suite. The whole suite of rooms should be made with impervious floors, walls and ceilings to allow thorough cleaning

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and fumigation, if required. Unnecessary clutter should be kept away, but there must be adequate space for all the equipment and instruments needed.

A procedure suite should have three distinct areas, as follows.

1. Preparation area, where animals are anaesthetised and then prepared for surgery. This is the least clean area within the surgical suite.

2. Operating area, used only for surgery. This is the cleanest area and needs careful isolation from the preparation area to avoid contamination by airborne dust and hair from clipping.

3. Recovery area, where animals are observed and monitored until fully conscious and ready to be returned to their normal cage or pen. This needs a high standard of cleanliness with good lighting and ease of observation. Intensive nursing care may be needed, and appropriate equipment must be available. This room needs to be easily accessible for people, unlike the operating room.

Each should be stocked with equipment appropriate for the tasks done in each, and prepared before use.

Personnel

First, check that all legal requirements are met before starting any procedure. Then all staff involved should be trained and competent in the procedure. *The performance of surgical procedures requires a high level of manual skill that can only be acquired by extensive practice.* In the UK practice is usually restricted to performing procedures on dead animals or simulators, such as the CurVet rat training siumulator (see www.learningcurvedevelopment.co.uk/training-materials/). Prepare by consulting text books or DVDs, observing and discussing with others, learning the relevant anatomy, practising on cadavers or simulators and then operating under direct supervision initially. Whenever possible, obtain practical instruction from someone who has undergone formal surgical training (a medically qualified or veterinary surgeon).

Liaise with support staff to make sure that they will be available, and it may be helpful to prepare a peri-operative plan in consultation with the animal technicians and veterinary surgeon, to include husbandry requirements, post-surgical welfare assessment and a protocol for analgesia. Do not attempt any surgical procedures on live animals, however minor, until you are properly prepared and confident of your ability to carry it out successfully.

Principles of Aseptic Technique

Infection is one of the most significant potential adverse effects from surgical procedures. Precautions must therefore be taken to prevent infection arising from contamination of the wound during surgery. Animals have no ‘innate resistance’ to infection and wound infections do occur, leading to clinical or subclinical effects on the animals[2]. Infection leads to inflammation, pain, wound breakdown and delayed healing. This is detrimental to animal welfare and results in poor science as results may be confounded by infection or treatment. For most wound infections the source of the pathogen is the animal's skin, mucous membranes or bowel and rarely from another infected site in the body. Exogenous sources of pathogens (surgical team, instruments and equipment, etc.) are occasionally responsible.

During surgical procedures, bacteria and other microorganisms contaminate all surgical wounds, but only a small number of patients actually develop a clinical infection[3]. In most cases infection does not develop because host defences eliminate the contaminating organisms. Whether infection develops depends on several factors, for example:

- number of bacteria entering the wound,
- type and virulence of the bacteria,
- bacterial population: mixed infections can potentiate infections,
- effectiveness of host defence mechanisms: immune-compromised animals are commonly used in research and are more susceptible to infection,
- duration of exposure: risk increases with procedures lasting more than 4 h,
- bacterial requirements for growth: oxygen and nutrients are needed.

Two factors that can minimize the number of organisms entering the wound are the skill of the surgeon and use of good surgical technique. If a surgical site is contaminated with more than 105 (100 000) organisms per gram of tissue, the risk of infection is markedly increased[4]. The dose required for infection can be even lower, however, in some tissues (bone, central nervous system tissues), in immune-compromised animals, or if foreign material is present at the site. About 100 staphylococci per gram are

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enough if silk suture is used for closure or to control bleeding[5]. Although the type and virulence of the bacteria cannot be controlled, the other factors can, to a large extent. For example, the effectiveness of the host defences depends to a large extent on the animal's general health, age and immune status.

Aseptic technique describes the methods used to minimize contamination of the surgical site, by controlling the potential sources of contamination. These are the atmosphere, the surgical team, the instruments and equipment, and the skin of the animal. The rigor with which aseptic technique is applied dictates the ultimate outcome: procedures are either aseptic or not, there is no in-between. Careful preparation minimizes the risk of wound contamination and the development of infection, and is the most effective way to prevent post-surgical wound infection. The use of prophylactic antibiotics is *not* a substitute for good aseptic technique.

Aseptic technique should be used in all recovery surgery and lengthy procedures carried out under terminal anaesthesia. The Laboratory Animal Science Association (LASA)1 recommends that terminal procedures lasting more than 1 h should be carried out using aseptic technique. Whereas full aseptic technique should always be the goal, the methods used are influenced by the circumstances under which surgery is undertaken and the species involved. However, not using full aseptic technique will increase the likelihood of post-operative wound infection developing.

Atmosphere

Contamination from dust and airborne particles settles in wounds to produce infection. To control this, dust and air movement need to be minimised. There should be no clutter in the surgical area, with no open shelves and minimal fixtures and fittings. Fittings such as cupboards and ceiling lights should ideally be flush with walls or ceilings. The surgical suite should be maintained to a very high standard of cleanliness: all surfaces should be regularly cleaned and disinfected.

To reduce air movement, the ventilation should provide filtered air, so particles which may carry microorganisms are removed. The room should ideally be held at positive pressure, to push dust particles out of the room away from the animal. There should be no ceiling fans, and the room should be used only for procedures, and not be used for storage or as a thoroughfare. A laminar-flow cabinet may be used to provide a localised microclimate of filtered air in which to carry out surgery of small animals.

Surgical team

Before entering the surgical area, change outer clothing and put on a clean gown or scrub suit, hat and mask and overshoes or theatre shoes. Trim and clean under nails, remove jewellery and tie long hair back. For all sterile surgical procedures the operator should scrub up, or use an antiseptic rub containing both alcohol and chlorhexidine, which can be as effective if used correctly[6].

Protocol for scrubbing up

1. Wash hands, fingers (including nails) and forearms including elbows with antibacterial skin cleanser (chlorhexidine or iodine-based) and warm water.
2. Rinse, holding hands above elbows to prevent dirty water running to clean areas.
3. Repeat the wash, this time extending only to mid-forearm level.
4. Rinse again.
5. Scrub for 1 min with sterile scrubbing brush. Scrub the palms of the hands, all surface of the fingers including nails.
6. Rinse
7. Wash again, this time only hands and wrists, and rinse.

Depending on the procedure, it may or may not be necessary to wear a sterile gown, but sterile gloves should always be worn. Once the surgeon has prepared for surgery, non-sterile objects must not be touched, and if they are, gloves should be replaced with a new sterile pair. It is useful to have a 'non-scrubbed' surgical assistant to help during procedures, so the surgeon does not have to break sterility in order to adjust the anaesthetic or open additional instrument packs. Non-scrubbed assistants must not touch sterile areas. If no assistant is available, sterile foil or plastic can be used to cover adjustable knobs, for example on the anaesthetic machine or microscope.

Instruments

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Instruments and equipment to be used must be sterilised beforehand, then kept sterile throughout the procedure. Instruments can be sterilised in advance, then stored until needed. Prior to surgery they should be placed on to a sterile instrument tray and covered with a sterile drape.

There are many ways to sterilise instruments. *Sterilisation* is the removal or destruction of all pathogens and their spore forms. *Disinfection* is surface cleaning, killing most pathogens but not all bacterial spores. Methods that result only in disinfection are not adequate for preparation of surgical equipment.

There are three stages in the preparation of surgical instruments and equipment: clean, wrap and sterilize. After use, instruments must be washed thoroughly, to remove grease and gross contamination, which act as a barrier to the penetration of chemical and physical sterilising agents, paying particular attention to joints and teeth, where debris collects. Proprietary instrument cleaners are available for this purpose. Then they should be wrapped in a suitable material prior to sterilization. Commercially available plastic or paper wrapping materials are available, which allow steam or chemicals to penetrate, but protect the contents from microbiological contamination, and can be used for short-term storage of items after sterilisation.

Methods of sterilisation

Physical methods of sterilisation

Heat is the main physical method of sterilisation, either wet or dry.

1. *Autoclaves.* These utilise steam under pressure, and sterilise if used correctly. The time, pressure and temperature used vary with the material, with delicate materials needing lower temperatures and pressures but for longer periods than robust materials. The steam used in autoclaves penetrates through fabrics, so this method is useful for drapes, gowns, swabs and dressings. Autoclaving is generally not suitable for catheters, endoscopes and delicate materials. Items to be autoclaved may be free in the autoclave, or prepacked into autoclave bags made of plastic, paper or cloth. Several rules must be followed when using the autoclave, as listed here.

a. Steam must first displace the air before the sterilisation cycle begins.

b. To allow steam to penetrate, wrappings must be of suitable permeable material, fabrics must be folded loosely and not tightly packed, and the autoclave must not be too full, or it will not run efficiently.

c. An indicator should be used *within* the pack to check that the sterilisation process has been effective, not just on the outside. Indicator tape or strips are available which change colour when the process has worked.

d. After sterilisation, instruments should be allowed to dry, to prevent rusting.

e. Once they leave the autoclave, packets containing instruments have only a limited shelf-life during which they remain sterile. The date of autoclaving should be written on each packet and old packets re-sterilised at intervals.

Boiling only produces disinfection, and is unsuitable for routine preparation of surgical equipment.

2. *Dry heat.* Hot air ovens held at 160°C for at least 1 h will sterilise items. This is suitable for some metal and glass, and sharp optical instruments, as it does not cause blunting corrosion or pitting, which can occur with repeated autoclaving. The method is efficient, but slow. Also, instruments can be sterilised coated with grease, as this does not prevent the object from being heated. It is not suitable for fabrics and paper.

Hot bead sterilizers are useful when performing minor procedures where several animals have to be prepared at the same time. They contain a reservoir of glass beads, which can be heated up to 260°C. The instrument is inserted into the beads, and the portion of the instrument in the beads can be sterilized in as little as 15 s. Instruments must be allowed to cool before use, and the portion of the instrument which is not in contact with the beads is not sterilised by this method.

3. *Ionising radiation* using gamma rays is used commercially for sterilizing consumables, and delicate items. Items are then purchased pre-sterilized, ready for use. Such items have an expiry date and sterility of the contents cannot be guaranteed after this date.

Chemical methods of sterilisation

Chemical methods are less efficient than physical means, but are used for delicate items. Instructions must be followed carefully for good results. Several factors must be taken into consideration before using chemical sterilising agents.

- Items to be sterilised must be very clean or the chemical will not penetrate. Many chemicals are inactivated by debris,

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such as blood.

- The method is slow, and sufficient time must be allowed for sterilisation to occur.
- Liquid sterilisers must be at the correct concentration. A higher concentration does not necessarily mean more efficient sterilisation.
- Most chemical agents are irritant, so all residues must be removed prior to use of the instrument.
- For gaseous agents, items must be dry and moisture free.

Chemical agents are often used as quick sterilising agents in field situations, where there is no access to an autoclave, however most cold sterilisers require 10–12 h contact time to be fully effective. Disinfection may take place in 10 min, and this can be useful in emergency situations.

- *Phenolics.* Phenols and cresols are effective disinfectants, but are poorly sporicidal even at high concentrations, although sporicidal activity is enhanced at high temperatures[7]. If instruments are cleaned of gross contamination and then immersed for 1–2 min they will be disinfected.
- *Glutaraldehyde.* This is available in solutions of 1–3.4%, disinfects in 3–4 min depending on the concentration and sterilises in 12–24 h. It is useful for optical instruments.
- *Ethylene oxide.* This is a potent and effective way to sterilise items that will not withstand autoclaving. It is inflammable and explosive when mixed with air, so it has to be used with care in a sealed chamber. Usually it is mixed with nitrogen or carbon dioxide. Most ethylene oxide sterilizers involve three different stages: preconditioning, sterilization and degassing. During preconditioning, temperature and humidity are controlled to allow microorganisms to grow. Then, during the sterilization phase, ethylene oxide is added. The gas stays in the heated chamber for a defined period of time before it is flushed. After sterilisation there is a prolonged aeration stage to ensure all traces of the ethylene oxide are eliminated, or there will be severe tissue reactions. Due regard must be paid to health and safety regulations on its use because of its toxicity and irritancy.

Immersion in 70% ethanol does not sterilise even after several days' immersion. Ethanol is also unsuitable for use as a surface cleaner, because rapid evaporation makes adequate contact time impossible on open surfaces[8].

Preparation of the animal

The body of the animal carries two sorts of contaminant: resident commensal or symbiotic flora, and extraneous potential pathogens. To minimise the risk of contamination from these pathogens, the animal should be prepared in three stages: clip, clean and drape.

Clip

Prior to surgery, animals covered with fur need to be clipped to allow cleansing of the skin. This may cause trauma to the skin and must be done with care. Note that clipping, not shaving with a razor, is recommended, as shaving is associated with increased skin damage and post-surgical infection[9]. In any case, care must be taken to remove any loose hair. Clippers need to be suitable for the size of animal and maintained in good condition. They should be cleaned and disinfected after each use. If this is not done, they start to pull the hair and can cut the skin, particularly in animals with delicate skin, such as rabbits, and around delicate areas. An adequate area must be shaved to prevent contamination of the wound by adjacent fur, but too much fur removal will contribute to heat loss in smaller animals during and after surgery.

Clean

After clipping, the skin should be cleaned with a non-toxic antiseptic agent, to kill pathogens or arrest their multiplication.

There are three stages in preparation of the skin.

1. A wash to remove any gross debris, such as mud or faeces. Be sure to use a cleaning agent that is compatible with antiseptic agent you will be using.

2. An antiseptic wash, with a quaternary ammonium compound or an organic iodine preparation. Chlorhexidine is more effective and persists for longer[6,10]. Using clean swabs, the skin should be cleansed from the middle of the clipped area towards the outside, never the other way round, or this will drag dirt from the fur at the edge onto the area where the incision will be. This should be repeated at least three times. It is particularly important with small laboratory animals to avoid hypothermia, so solutions used for preparation of the skin must be warm and the fur must not be allowed to get

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overwet.

3. A final skin prep of alcohol may be applied to the skin. Again, in small animals this may be omitted to prevent excess heat loss.

Drape

After the skin has been cleaned sterile drapes should be applied to cover all areas except the surgical field. Drapes separate the sterile surgical site from contaminated areas of the animal, and prevent inadvertent contamination of instruments. Fabric, paper or plastic drapes may be used, and may be self-adhesive, or secured with towel clips. Drapes must be dry, or they act as a wick, drawing contamination up from beneath and onto the surgical site.

Positioning

The animal must be appropriately positioned for surgery. Extend the head and neck and ensure there is no restriction to respiration. There is a variety of commercial cradles available for positioning animals. Cheap animal-positioning devices can be made from pieces of foam rubber or sand bags, which can be covered in washable material, and these also provide some insulation around the animal reducing heat loss. If the procedure is to be prolonged, bony areas should be padded to prevent pressure sores, and bland ophthalmic ointment put into the eye to prevent corneal drying. In ruminants, a stomach tube should be passed to prevent ruminal tympany.

Summary of Lasa Guidelines on Rodent Surgery

- Rodents are not immune to infections and the aim should be to minimise contamination as much as possible.
- Always use sterile instruments – but a hot bead steriliser may be used between animals for batch surgeries. Keep instruments on a sterile drape or tray.
- Always wear new sterile gloves for each animal.

Conduct of Surgical Procedures

Inflammation and wound healing

Good surgical technique requires an understanding of tissue damage and healing, in order to optimise conditions for healing. Any injury to living animal tissues causes damage to cells. Damaged cells release mediators, which result in the accumulation of inflammatory cells and humoral factors such as antibodies at the site of injury. This process is known as *inflammation*. These cells and humoral factors begin the process of healing. Inflammation produces several characteristic clinical features. These are as follows:

- *heat* (*calor*): the affected area becomes warm to the touch,
- *redness* (*rubor*): the tissue becomes red (non-pigmented areas) due to dilation of blood vessels,
- *swelling* (*tumor*): the infiltration of cells and fluid causes the area to swell,
- *pain* (*dolor*): inflammatory mediators stimulate nerve endings to cause pain; some analgesics work by blocking the release of these inflammatory mediators,
- *loss of function* (*defunctor*): voluntary or involuntary restriction of movement.

After injury, changes occur in the tissues which result in inflammation and begin healing. The sequence of events is as follows.

Inflammatory phase

1. *Haemorrhage*: bleeding from damaged blood vessels occurs and is then arrested by the aggregation of platelets and fibrin, which form a scab.

2. *Inflammation*: mediators from damaged cells cause the influx of cells and humoral factors.

3. *Primary wound contraction*: local fibroblast cells contract to reduce the area of the wound as much as possible. This

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phase lasts 3–5 days.

Proliferation phase

1. *Epithelial proliferation*: this begins within 12–24 h. The cells divide and migrate over the surface of the wound. A moist environment facilitates this.

2. *Granulation*: fibroblasts and capillaries beneath the epithelium begin proliferating after approximately 36 h. They form granulation tissue, which is bright pink, firm, flat and resistant to infection. It fills in any defects in tissues and provides oxygen for the proliferating epithelium and surrounding parenchymal tissues. Granulation tissue will only form once inflammation has subsided and the area has been cleared of necrotic debris by macrophages.

It is normal for the epithelium to heal before granulation has filled in the defects in deeper tissues, leaving a concave surface. Granulation will continue beneath the surface until the surface is flat. This stage can continue for 4–6 weeks.

Maturation phase

Local fibroblasts lay down collagen, and granulation tissue matures into fibrous tissue to form a scar. This area then undergoes secondary wound contraction or cicatrisation, which reduces the area of the scar, but can result in the restriction of movement or the occlusion of a hollow organ. Initially, old collagen at the wound edges softens before sufficient new collagen is laid down to increase wound strength after 5–7 days. Wound strength continues to increase for up to 2 years depending on the nature and site of the injury.

Remember that wound edges swell due to inflammation and weaken in the first few days post-trauma, so sutures must not be too tight or too close to the edge, or they will cut into the tissues in this period.

Types of healing

There are several possible outcomes following healing, depending on the nature of the injury and the tissue type.

- *Resolution*: this occurs when there is minimal tissue damage. The inflammatory exudate and cells are resorbed with no tissue destruction or scarring, and the tissues return to normal. This occurs after minor injuries such as bruises.
- *Regeneration*: areas of lost or damaged tissue are replaced by differentiation of surrounding tissues until the organ mass has returned to normal, with no permanent loss of function. There may be disruption to the architecture of the organ. Bone and liver heal this way.
- *Suppuration with tissue death*: if there is much tissue damage, areas of devitalised tissue undergo necrosis and liquefy, with or without the presence of infection. The purulent exudate formed is released by sloughing or tracking along lines of low resistance to the outside. The defect produced will eventually heal by granulation and scarring, once the exudate has been cleared.
- *Repair by fibrosis*: lost tissues are replaced by fibrous tissues to form a scar. Scar tissue is non-functional, and tends to contract, which can lead to problems as outlined above.

Factors affecting healing

Healing is affected by a number of factors, both local and general.

Local

- *Infection*: bacteria consume oxygen, attract inflammatory cells and mediators and inhibit epithelialisation and wound contraction.
- *Tension and pressure*: excess tension or pressure interferes with nutrition of the wound edges.
- *Interference with blood supply*: a satisfactory blood supply is required for healing.
- *Presence of alien tissues*: for example, pieces of fat between skin edges or neoplastic cells in any wound prevent healing.
- *Persistent irritation*: hair around the wound edges or interference from the animal or its cage mates may prevent healing.

General

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- *Age*: healing tends to be slower in older animals.
- *Nutritional status*: protein malnutrition in particular leads to poor healing.
- *Corticosteroid level*: these hormones are released during stress, and affect the function of phagocytic cells and reduce collagen formation.
- *Reduced tissue oxygen and intercurrent disease*: disease may reduce tissue oxygen, cause stress or affect nutrition, all of which affect healing. Experimental animals should be disease free.
- *Region of body and tissue type*: different tissues have different blood supplies and, therefore, different oxygen levels. Tissues such as muscle and epithelium have good blood supplies and heal quickly, whereas fascia and connective tissues have poor blood supplies and heal slowly.

Many of these are under the control of the person performing the surgery. The amount of pain, suppuration, fibrous tissue and scarring produced depends on the degree of tissue damage and inflammation produced. Good surgical techniques will result in minimal production of inflammation, infection and scarring, and will improve healing. Genetically modified animals may have unknown effects on immune function or healing, so particular care may be needed with these animals.

Performance of surgical procedures

Surgical procedures must be performed competently, to cause the minimum amount of trauma to the tissue and facilitate rapid healing. Instruments must be handled correctly to avoid damaging tissues, by grasping them with excessive force, and must be a suitable size for the species and operative site. Before starting any surgical procedure, count the swabs and instruments to be used, paying particular attention to small items. Then, when the wound is about to be closed, check carefully that everything has been accounted for and nothing has inadvertently been left inside the animal.

William Halstead[11] devised a set of principles of surgery in the 1890s, and these still form the basis of good surgical technique. These are:

- handle tissues gently,
- ensure meticulous haemostasis,
- preserve the blood supply,
- ensure strict asepsis,
- apply minimal tension to tissues,
- ensure accurate tissue apposition,
- obliterate any dead space.

Careful technique and adherence to these principles will result in minimal damage and optimal healing. Luck is not a factor: good techniques will produce good results.

Surgical procedures should always be kept as short as possible, as post-operative infection is related to the length of the procedure, and care must be taken to stop exposed tissues from drying out. Tissues should be protected from drying by covering them with sterile gauze swabs soaked in warmed saline or by direct applications of warmed sterile saline. In general, try to touch tissue that is to be left in situ as little as possible, holding it with atraumatic instruments if necessary, and only use crushing instruments on tissue that is to be discarded.

Any surgical procedure consists of four components: incision of the skin and body wall, the dissection of deeper tissues, haemostasis and wound closure. *Anything you cut has to be repaired*.

Skin incision

To gain access to underlying tissues, incisions must be made in the skin and, if going into a body cavity, the body wall. Usually the skin incision is made using a new scalpel blade. A scalpel causes minimal damage to tissues, as it cuts without crushing, and leads to optimal healing. The muscle and connective tissue of the body wall should then be incised using round-ended scissors, to avoid damaging structures underneath. Scissors should not be used to cut skin, since they cause more trauma and delay healing. Diathermy can also be used to incise vascular tissues. This uses an electrical current and heat to cut and cauterise at the same time. Bleeding is minimal, but significant trauma is caused compared with a scalpel, so again healing will be delayed. Haemorrhage from the skin edge comes from capillaries and occasionally from small arteries and veins, and firm pressure with a sterile swab is usually sufficient to stop this, although specific vessels may require diathermy, ligatures or application of artery forceps. Skin may be handled with fine rat-toothed forceps, which grip positively but with minimal trauma, or tissue forceps such as Allis tissue forceps may be used.

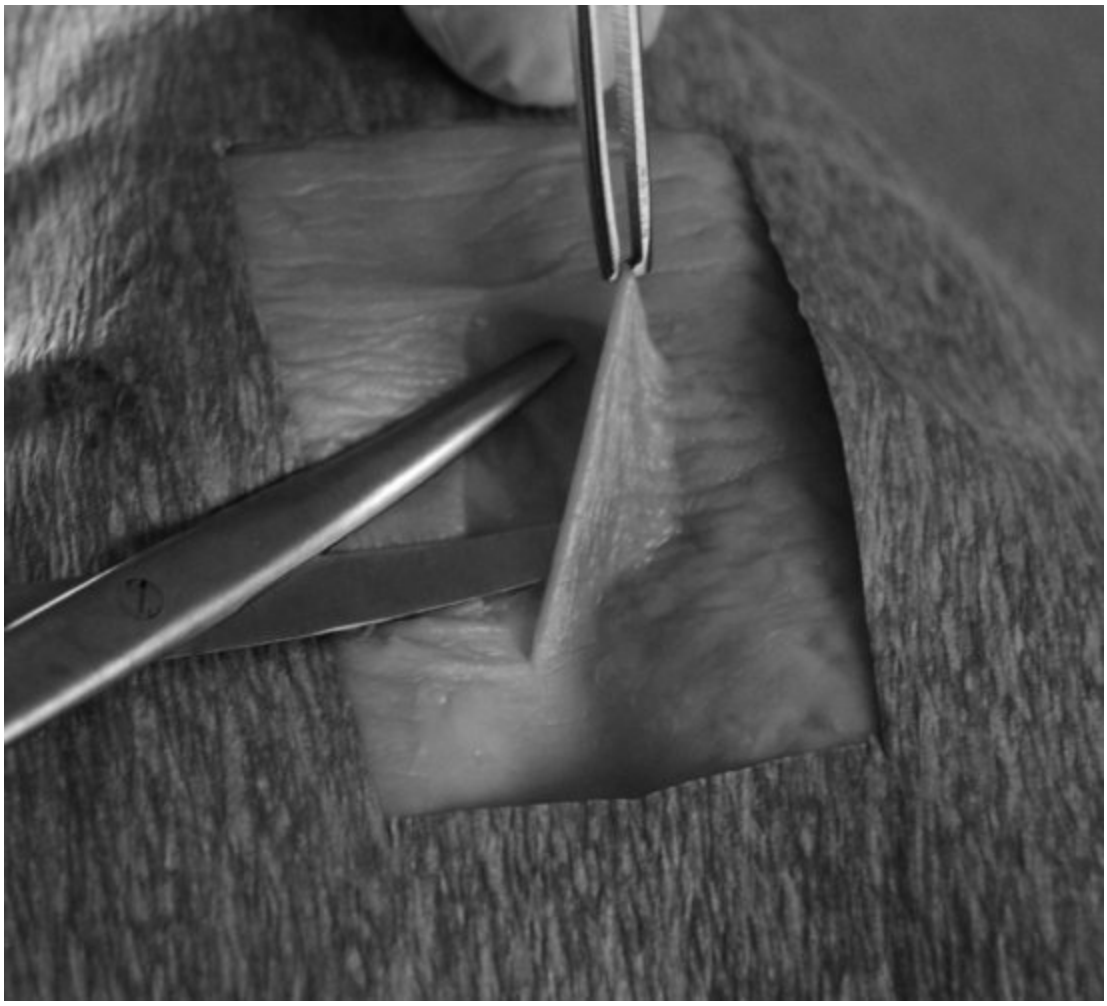
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In order to avoid bruising, tearing and stretching of tissue it is preferable to make a larger, rather than a smaller incision. Wounds heal from side to side, not from end to end and, therefore, a larger incision, which allows adequate vision, is always better and will not have any effect on the rate of healing. An incision made with sharp instruments with minimal handling of tissue and sutured appropriately will heal rapidly with minimum discomfort to the animal.

Dissection

Having cut through the skin, deeper tissues may need to be held or separated to gain access to the organ of interest. Tissues may be separated either by sharp or blunt dissection. *Sharp dissection* involves cutting tissues with scissors or a scalpel. This carries a significant risk of damage to blood vessels or nerves, and is best only used on tough structures once they have been clearly identified. *Blunt dissection* separates tissues along natural cleavage planes, with minimal risk of damage to nerves and blood vessels, and it fulfils the need to handle tissues gently. Round-ended scissors are used for this. They are inserted into the tissue plane closed, then opened. As the blades separate, tissues are pulled apart gently without damaging adjacent structures. Fingers may also be useful for blunt dissection, or 'paintbrushing', which involves using a sterile cotton bud or a gauze swab wrapped around the jaws of sponge-holding forceps to wipe away fatty connective tissue from structures. See Figure 10.1.

FIGURE 10.1 Blunt dissection. Round-ended scissors are inserted closed between the tissue planes and then opened, separating tissues along natural cleavage planes.



Fatty tissue must be handled gently during procedures, as if crushed it is prone to liquefactive necrosis. Since it has a poor blood supply, it heals slowly if damaged and is at risk of infection.

Fibrous connective tissue consists of a dense network of collagen and needs to be divided by sharp dissection. However, due to the proximity of nerves and blood vessels, this must be done with care and scissors may be preferable to use of a scalpel. Toothed

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forceps or tissue-holding forceps are needed to handle this tough tissue.

Muscle is very vascular and bleeds profusely when incised. If possible, use a surgical approach which avoids cutting muscles. If an approach has to be through a muscle, then it is better to split the muscle longitudinally between the fibres rather than cut it transversely, since this will not result in a significant loss of function once healing has occurred.

Haemostasis

Good surgical technique aims to prevent excess bleeding from a surgical site, but if haemorrhage occurs, there are several ways to arrest it.

If in doubt, apply pressure. Digital pressure using a swab for a few minutes can be sufficient to arrest even quite severe haemorrhage. Always dab or press rather than wiping, as this will remove the clot and allow continued haemorrhage. For small vessels, a clot may form to seal the hole within a few minutes. If not, bleeding vessels can be picked up and crushed using suitable artery forceps, and then tied off with a ligature using a fine suture material, or sealed using diathermy or cautery, all of which apply heat to the vessels to coagulate them. Large vessels usually require ligatures for secure haemostasis. Metal clips can also be used, but they require special instruments for placement. Ligatures for haemostasis should be made of absorbable materials since non-absorbable materials can act as a focus for infection.

The amount of blood loss should be carefully monitored, as a very small volume is potentially very significant in a small animal. Mice for example typically have a total blood volume of less than 2 ml. In larger animals it is useful to weigh the swabs used to give an estimate of blood loss, so fluids can be infused at an appropriate rate. Whatever the size of the animal, good control of haemorrhage is always important to avoid post-operative swelling and to minimise surgical shock and risk to the animal.

Surgical Instruments

Surgical instruments exist in vast numbers and varieties. The choice of instruments depends on the size of the animal, the organ system and tissues to be handled, availability, and the surgeon's preference. Some of the more common instruments are described here. Although there are no instruments designed specifically for rodents, ophthalmic instruments and fine-surgery kits may be used for procedures on mice and rats. A selection of different instruments will be required for different procedures – one size does not fit all – and choosing the right instrument can improve the outcome of a surgical procedure.

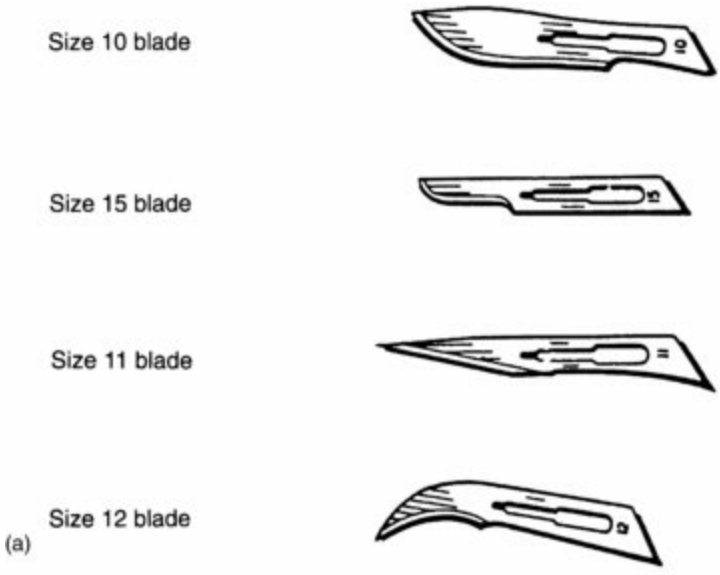
Good-quality instruments are always worth the cost, and they must be looked after to ensure they remain in good condition. Surgical instruments must be used *only* for surgery. A veterinary surgeon can advise on choice of instruments. Instruments will be needed for the different parts of the surgical procedure: incision, dissection, haemostasis and closing the wound afterwards. Instruments are designed to be optimum for particular tasks, and therefore appropriate instruments should be selected for different parts of the procedure. It is not advisable to use instruments designed for one purpose for another; for example, artery forceps are not suitable for holding suture needles, and rat-toothed forceps are not appropriate for holding delicate tissues.

Skin incision

Scalpels are very sharp, and cause the minimal amount of trauma, resulting in faster healing with less tissue damage and scarring. However, because there is less tissue damage, they also cause significant bleeding. Scalpels should be used for cutting through skin and tough tissue. See Figure 10.2.

FIGURE 10.2 (a) Scalpel blades. (b) Holding a scalpel.

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Scalpel blades are available in a variety of shapes and sizes, which are designed for different purposes. They may come individually wrapped and pre-sterilised, or non-sterile in packs. Before use, they should be attached to a suitable scalpel handle. Fixed-blade scalpels are also available.

- Size 10 blades are all-purpose blades with a rounded cutting edge, which can be used for most surgical procedures.
- Size 11 blades are pointed, with a straight blade, and are particularly useful for work involving fine cutting of connective tissue.
- Size 12 blades are curved with the cutting edge on the inside of the curve. They are useful for situations where the skin needs to be cut without damaging the structures beneath.
- Size 15 blades are a similar shape to size 10, but smaller and are useful all-purpose blades for fine work on small animals.

A scalpel should be held like a pen or a knife, and the tissue cut with a firm single stroke. The free hand should be used to tension the skin to ensure that the incision is straight and divides the full thickness of the skin in one stroke. When using size 10 or 15 blades, the wide round part of the blade should be used for cutting, rather than the tip.

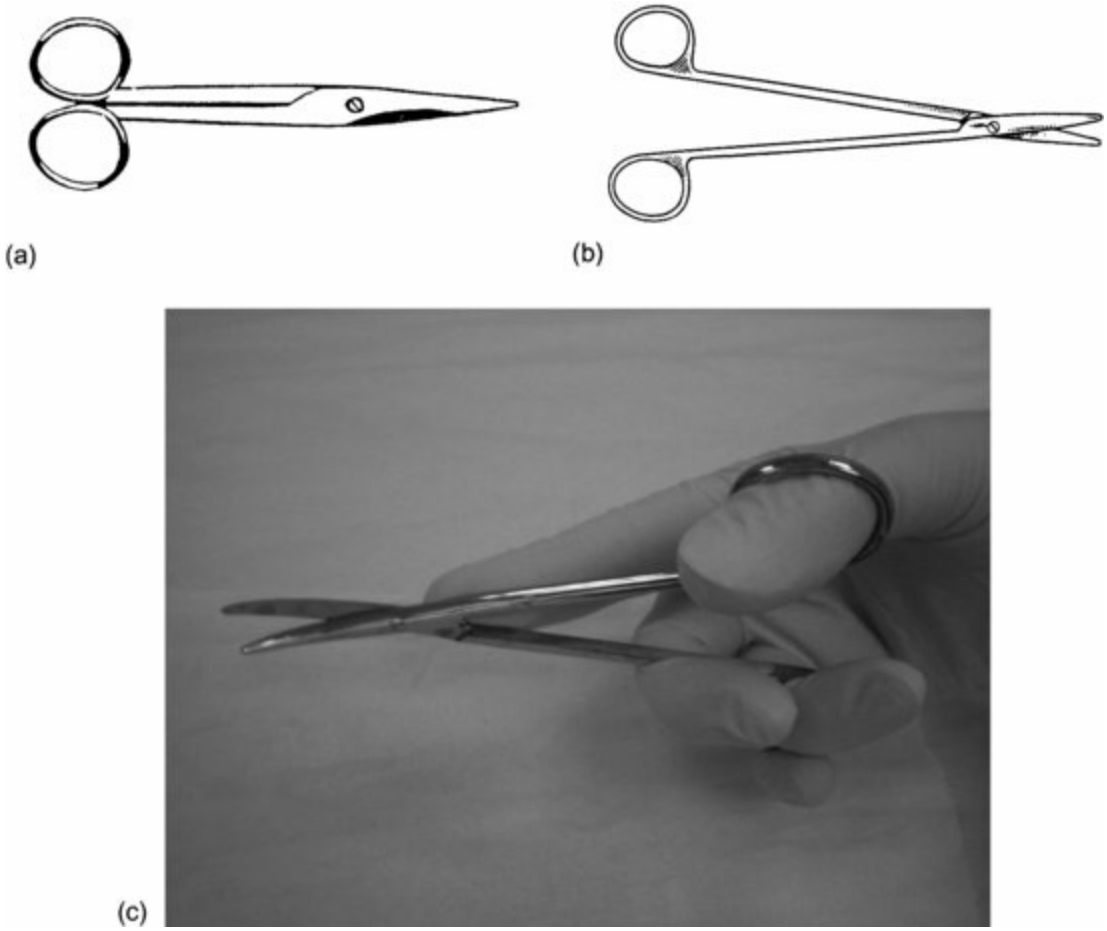
Round-ended *scissors* should be used to cut through the body wall. Pointed scissors should not be used for this, to avoid damaging structures beneath. Scissors should not be used to cut skin as they cause more trauma and bruising than scalpels.

Diathermy and *thermocautery* units use an electric current to cut the skin and coagulate blood vessels at the same time. They can also be used just for haemostasis. These also cause more trauma and scarring than scalpels.

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Scissors can be used for sharp or blunt dissection. Round-ended scissors are used for surgery, such as Mayo scissors. Fine-ended scissors are available for delicate work, for example Metzenbaum scissors. Scissors should be held with the ring or third finger through the loop and index finger close to the blades to allow fine control (Figure 10.3).

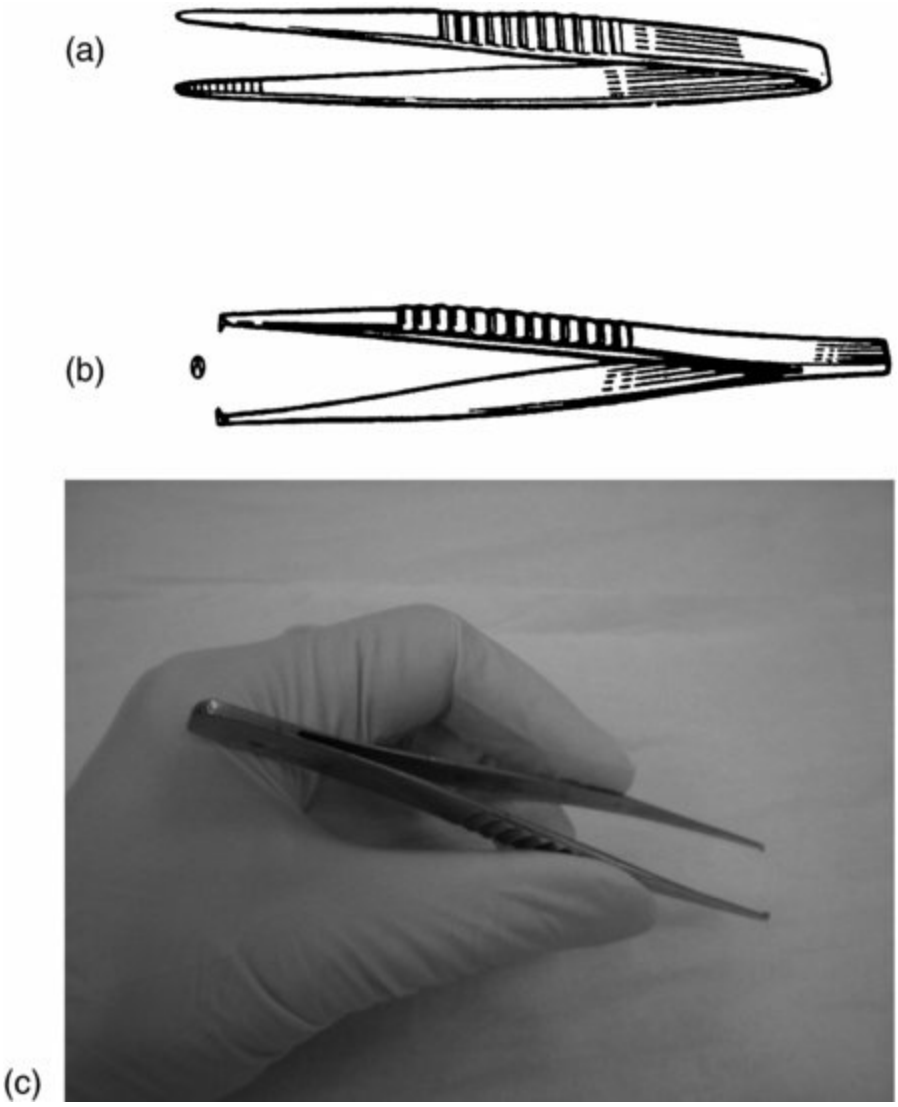
FIGURE 10.3 (a) Mayo and (b) Metzenbaum scissors. (c) Holding scissors.



Forceps are used to hold tissue, organs or vessels while carrying out blunt dissection or suturing. Rat-toothed forceps have teeth to grip tissue firmly and are used in skin and tough tissue. Blunt forceps have serrated tips and are used in delicate tissue. Forceps should be held like a pencil or a pair of chopsticks (Figure 10.4).

FIGURE 10.4 (a) Blunt and (b) rat-toothed forceps. (c) Holding forceps.

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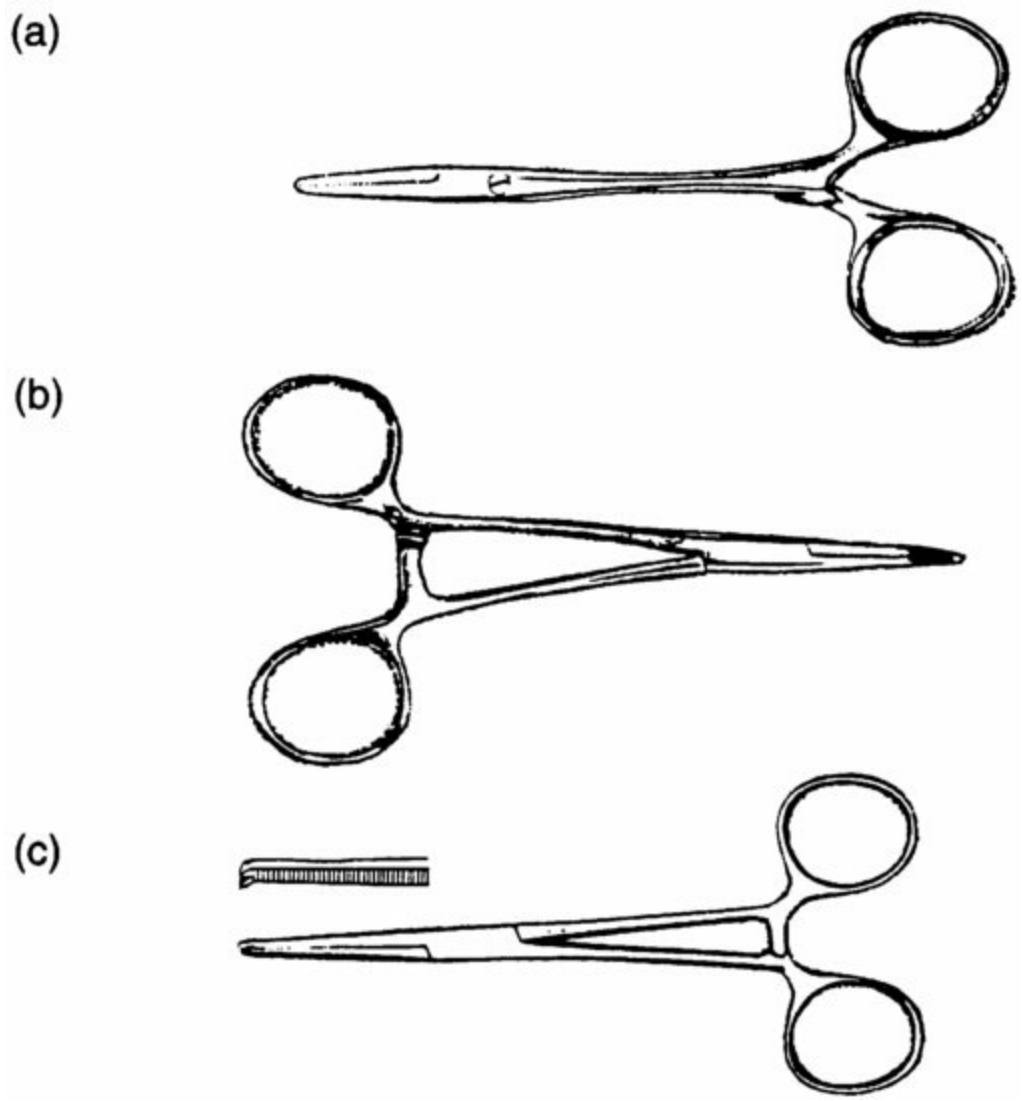
Haemostasis

Haemostatic or *artery forceps* are used to clamp and hold blood vessels. They can be curved or straight, and have crushing tips to trigger blood clotting by producing tissue damage. Artery forceps need to be in good condition with jaws that meet properly. Artery forceps need to be properly maintained and looked after to ensure that the jaws continue to close fully, and to that end they should not be used for any other purpose, to avoid damage to the instruments.

Spencer–Wells' and Dunhill artery forceps are general-purpose haemostats. Halstead's mosquito forceps are very fine, and are useful in small animals and for delicate work. Kocher's artery forceps combine a rat-toothed tip with crushing jaws, and are useful for grasping bleeding vessels deep within tissues (Figure 10.5).

FIGURE 10.5 Artery forceps. (a) Spencer–Wells' forceps. (b) Dunhill's forceps. (c) Kocher's forceps.

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Wound closure

Following a surgical procedure, wounds may be closed with needles and suture material, skin clips, surgical staples or occasionally tissue glue.

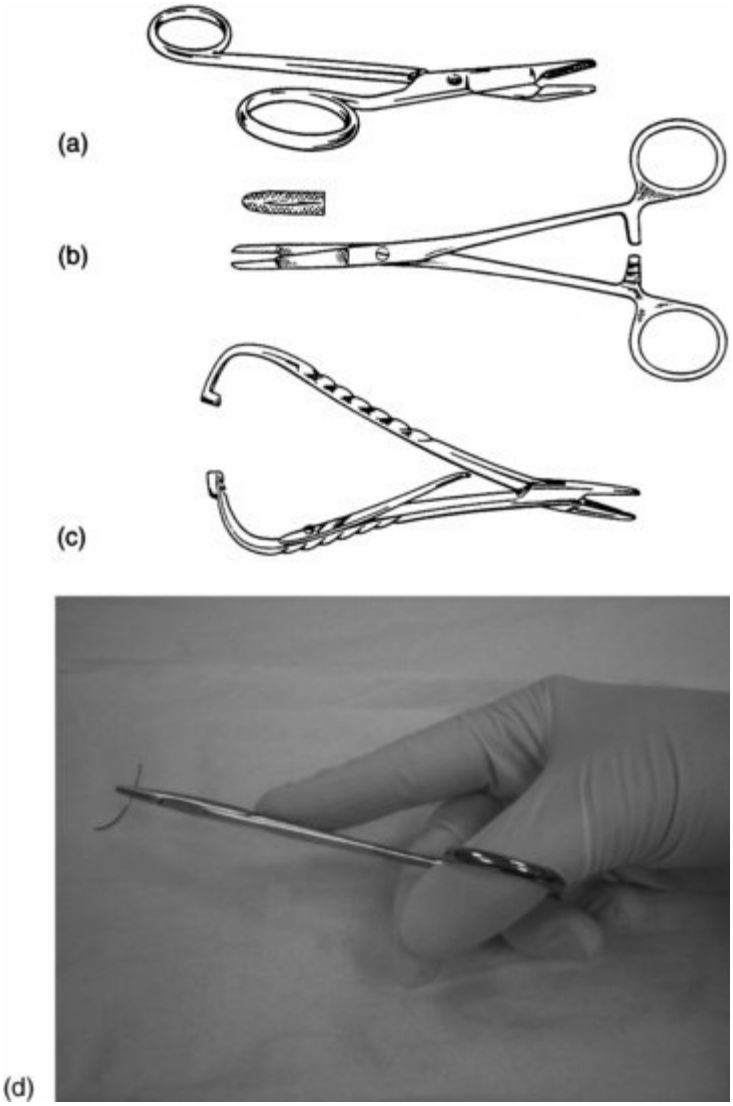
Needle holders are hinged instruments used to hold curved needles while suturing tissue. Straight needles are usually held by hand. Needle holders come in many patterns (Figure 10.6 and Table 10.1), and may come with or without a ratchet which locks the jaws onto the needle, with or without integral scissors so separate scissors for cutting sutures are not necessary, and with or without rings. To a large extent personal preference dictates which type is used. For needle holders without a ratchet, considerable digital pressure may be required to hold the needle if the tissue is tough.

Table 10.1 Needle holders.

Needle holder	Scissors	Ratchet	Rings	Notes
Gillies'	✓	×	✓	Right- and left–handed versions
Olsen-Hegar	✓	✓	✓	
MacPhail's	×	✓	×	
Mayo	×	✓	✓	

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FIGURE 10.6 Needle holders. (a) Gillies' needle holders. (b) Olsen-Hegar's needle holders. (c) MacPhail's needle holders. (d) Holding needle holders.



The jaws of needle holders are ridged such that rotation of the needle is minimised.

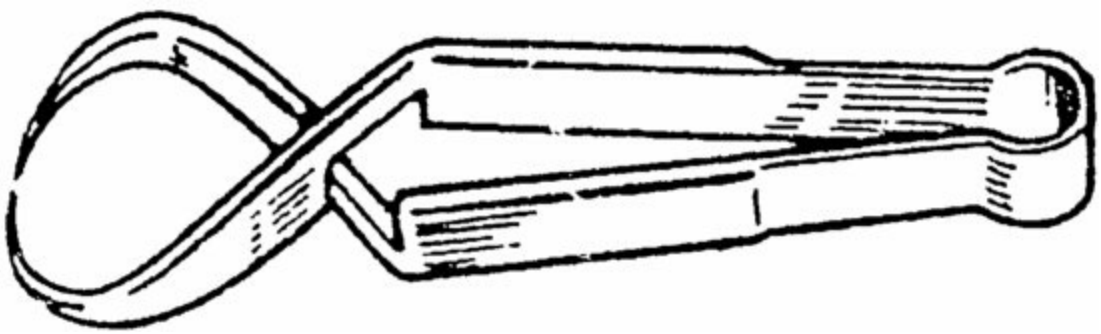
Other instruments

In addition, other instruments are available, designed for specific parts of the surgical procedure.

Towel clips are useful for securing paper or cotton drapes, so that surgical instruments and suture materials do not come into contact with non-sterile parts of the animal. They are available in a variety of patterns (Figure 10.7). Care must be taken in their use, however, since they have sharp teeth and can cause trauma if used incorrectly.

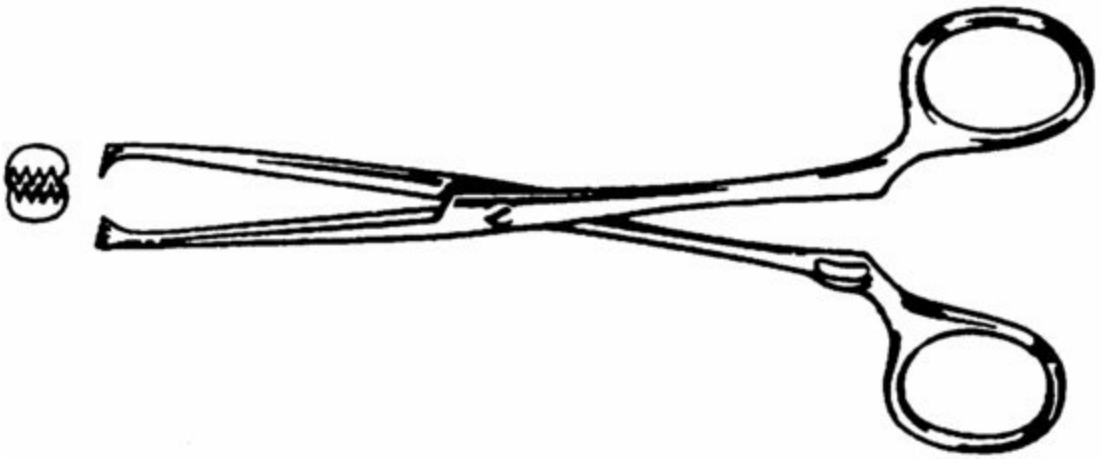
FIGURE 10.7 Towel clips.

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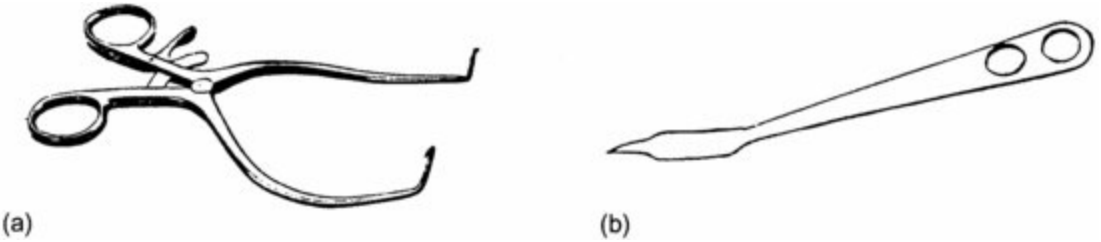
Allis tissue forceps are long forceps with small gripping jaws (Figure 10.8). They are useful for grasping pieces of tissue to hold them to one side temporarily, and cause relatively little trauma.

FIGURE 10.8 Allis tissue forceps.



Retractors are used to improve surgical access and visibility, and to hold tissues to one side. They may or may not be self-retaining. Many different varieties of retractors are available. Gelpi retractors have small hooks on the end, which are inserted between the tissues to be separated. As the handles are pushed together, the hooks separate forcing the tissues apart and the ratchet holds the retractor open. Hohman retractors are designed for lifting bones in orthopaedic operations, and need to be held by an assistant (Figure 10.9).

FIGURE 10.9 (a) Gelpi retractors. (b) Hohman retractor.



Atraumatic clamps: when performing vascular or intestinal surgery, it may be necessary to occlude a vessel or organ temporarily without causing damage. It is essential to use the correct atraumatic bowel or vascular clamp in these circumstances, which will occlude the lumen without causing damage to the wall of the organ.

Suturing Techniques and Materials

The purpose of any material used for wound closure is to hold the wound together for long enough for healing to occur. It should

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then either disappear or be removed. Each layer should be closed separately, to maintain anatomical relationships, and there should be careful approximation of tissues and obliteration of dead spaces. This can be done with sutures, surgical staples or tissue glue.

Sutures are generally placed using needle holders and tissue forceps. Needle holders come in many patterns and sizes (see above), and the choice will depend largely on personal preference. Whichever one is chosen, it is essential to know how to hold it properly and manipulate it with dexterity. Practice is essential, using cadavers, skin simulators, freshly incised banana skins or foam rubber. Suture pads designed to mimic the texture of skin are available from numerous sources (e.g. www.vet-tech.co.uk/home/index.html).

There are several different suture materials and needles that provide an accurate and secure approximation of the wound edges. Ideally, the choice of the suture material should be based on the biological interaction of the materials employed, the tissue configuration and the biomechanical properties of the wound[12].

All biomaterials placed within the tissue can damage the host defences and invite infection[13], and surgical needles have a proven role in spreading blood-borne infection[14]. Therefore, the operator must take all reasonable steps to reduce the risk of contamination or injury during surgery.

Suture needles

Suture needles can be curved or straight, cutting or round bodied, and swaged on or threaded.

Curved needles are used where there is limited accessibility of the operative site. The greater the curvature of the needle, the less control there is of the tip. Generally, the straightest needle possible should be used. Curved needles are always placed using instruments, whereas straight needles can be held with instruments or fingers.

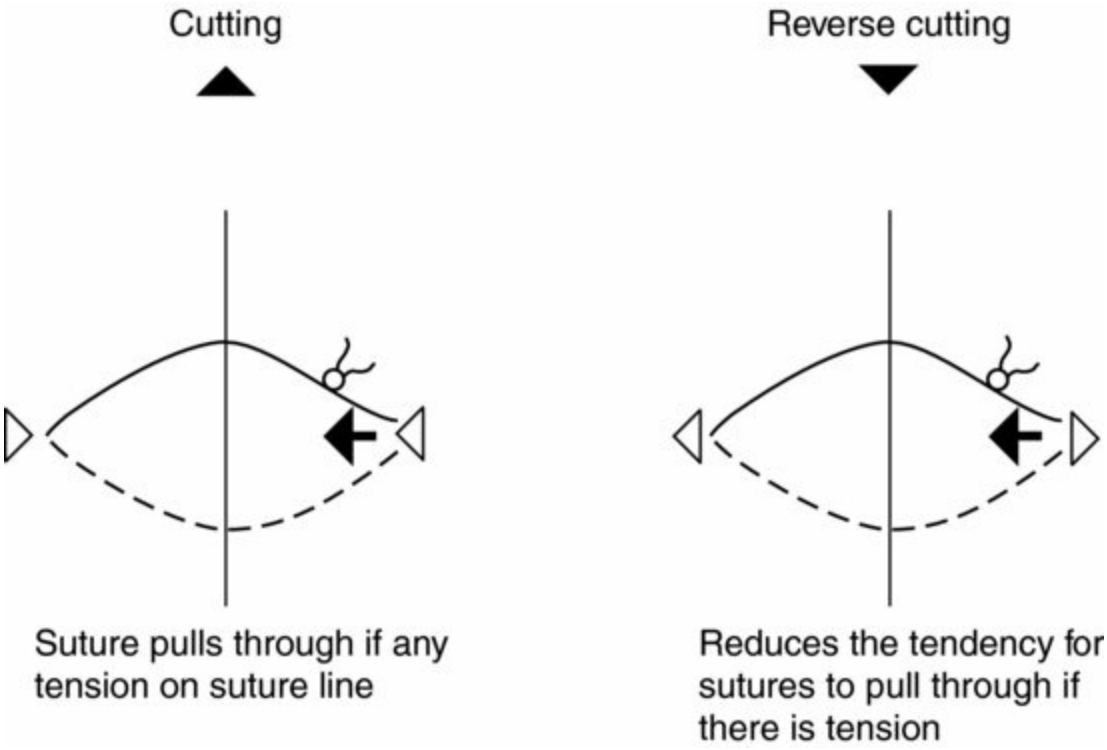
Suture needles may be round in cross section, or triangular, with three cutting edges. Round-bodied needles are atraumatic, and are designed to pass through tissues without leaving a hole, and as the needle passes through, the tissue closes tightly round the suture material forming a leak-proof suture line. They are used in soft tissues and in tissues where leakage must be avoided, such as bowel, blood vessels, internal organs and muscle.

Cutting needles are used in tough tissues, such as skin and fascia. Cutting needles leave triangular holes behind and should not be used in hollow organs as they encourage leakage of the contents.

Curved cutting needles may have the apex of the triangle on the inside of the curve, or the outside, in which case they are known as reverse cutting. With reverse cutting needles, the suture material is less likely to pull through the tissue, as the suture is not left sitting at an apex of the hole left in the tissues, and the needle has increased strength and resistance to bending (see Figure 10.10).

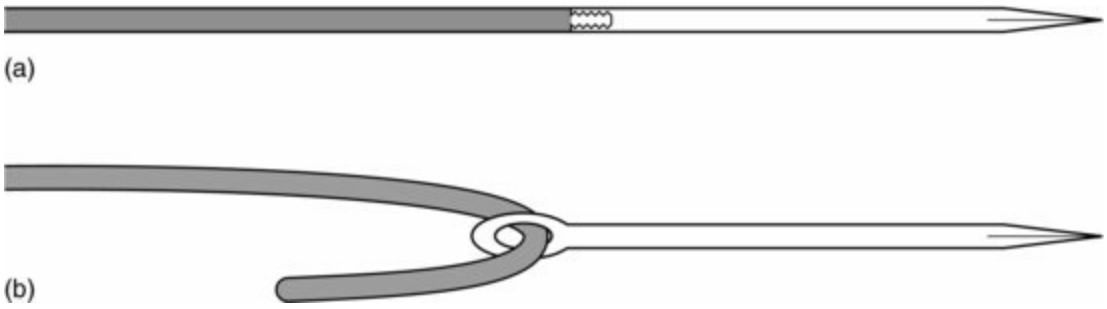
FIGURE 10.10 Cutting-edge needles for use in skin and fascia.

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Surgical needles may be swaged on, where the suture material is inserted into a hole in the end of the needle during manufacture, or threaded, with suture material being inserted through the eye of the needle immediately prior to use (Figure 10.11). Threaded needles have to be cleaned and re-sterilised individually before use, the suture material has to be cut from a reel which itself has to be kept sterile, two thicknesses of suture material have to be pulled through the tissues and the needles become blunt with repeated use, leading to increased tissue damage and impaired healing. Commercially available sutures with swaged-on needles are available pre-sterilised in easy-to-open packs, allowing the surgeon to utilise a new, sharp sterile needle and suture material each time. Only one thickness of suture material has to be pulled through the tissue, reducing tissue damage and improving wound healing.

FIGURE 10.11 Types of needle. (a) Swaged-on needle. (b) Eyed needle.



Suture placement

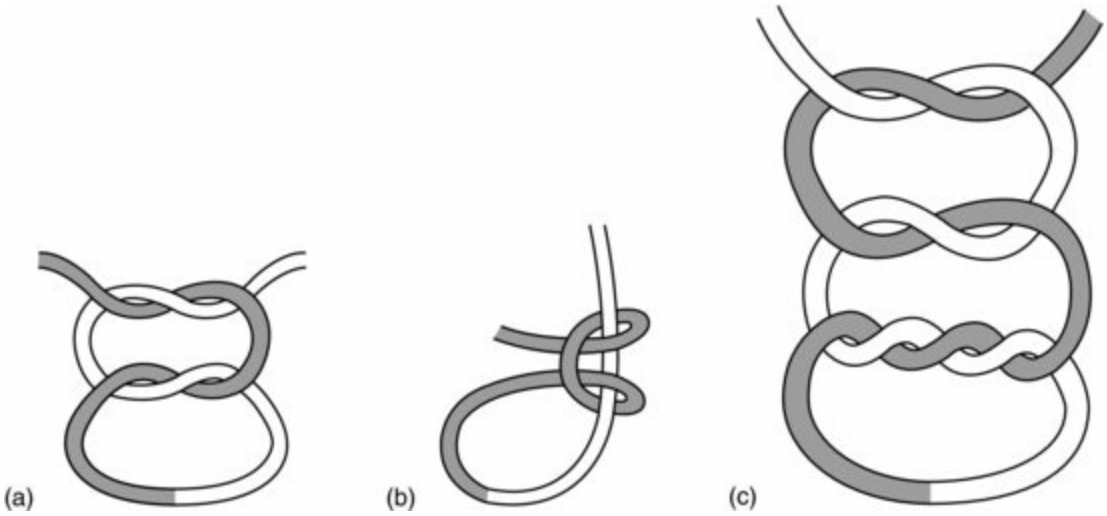
When placing sutures, the needle should be passed perpendicular to the tissue, to ensure any pressure exerted by the suture material on the tissue is spread out and does not produce pressure points. They should be placed sufficiently far from the wound edge that the sutures will not pull out: collagen at the wound edges softens in the first few days after an incision, and sutures placed close to the edge may have a tendency to pull through 3–5 days post-surgically. Similarly, sutures should be left slightly loose, to allow for post-surgical swelling. Otherwise they will become uncomfortable, causing the animal to chew at them, and are likely to pull through.

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Surgical knots

The basic *surgical knot* is common to all suture patterns. This can be hand- or instrument-tied. Essentially, it is a square (reef) knot with an extra throw; that is, right over left, left over right, right over left (see Figure 10.12a).

FIGURE 10.12 (a) The basic surgical (reef) knot. (b) Half-hitch (incorrect). (c) Surgeon's knot.

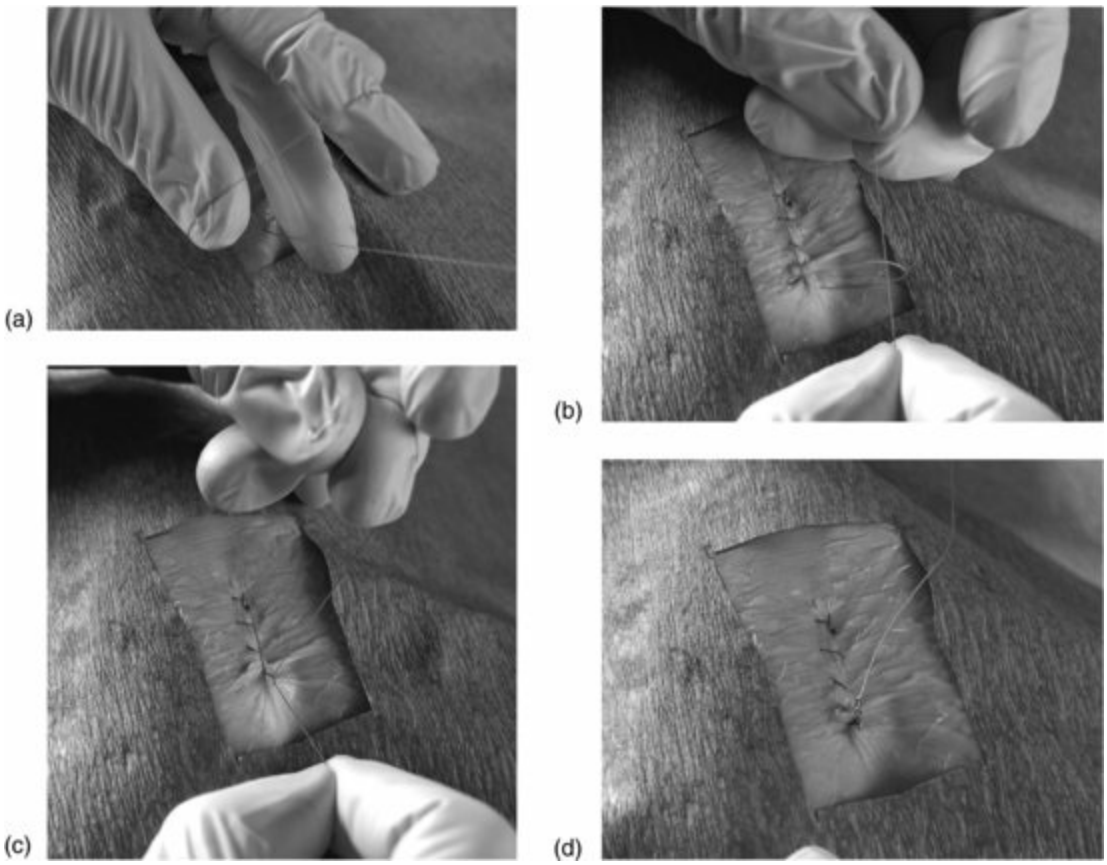


To tie a basic knot with instruments, first place the stitch across the incision. Notice that there is a long end of suture material with the needle attached, and a short end. Place the needle holders over the incision, between the long and the short ends of suture, and loop the long end of suture around the needle holder. Grasp the short end of suture with the needle holder, and pull it through the loop, moving the hands in opposite directions to form the first throw of the knot; note that the long end and the short end have changed places. The process is repeated in the opposite direction to form the second throw, making sure that both ends of the suture material are pulled evenly, otherwise half hitches may form, which are not secure (see Figure 10.12b). A third throw formed in a similar manner will lock the knot securely and prevent it from becoming untied. Three or four throws are sufficient for most suture materials. Additional throws are unnecessary; they add no strength and will simply add bulk and more foreign material, causing irritancy. Knot security is obviously important, and depends on the type of knot, the coefficient of friction of the suture material and whether the knot is tied correctly. For some slippery suture materials (some sutures are coated with wax or silicone to reduce tissue drag), or in wounds where there is some tension, special knots may be used, such as the surgeon's knot, where the first throw is a double one (see Figure 10.12c). The simplest knot that will provide security should be used, and the first throw should not be pulled so tight that the tissue becomes strangulated.

An alternative knot, which is particularly useful when placing a continuous suture, is the Aberdeen knot. At the end of the suture line, a loop of suture material is left when placing the last suture. A second loop of suture material is made from the long end of suture, and passed through the first loop. This is then pulled such that the first loop is pulled tight. A third loop is made from the long end, passed through the second loop, and the second loop pulled tight. This is then repeated five or six times. Finally the needle is passed through the last loop, and pulled tight. This produces a secure knot provided a minimum of three throws is used[15]. See Figure 10.13.

FIGURE 10.13 Tying the Aberdeen knot. At the end of a continuous suture, a loop of suture is left. (a) The loop is held between the index finger and thumb of the left hand. (b) The free end is grasped between the index finger and thumb of the left hand through the loop. The free end is pulled while releasing the right hand thread, to create a new loop. (c) The new loop is pulled until the first loop is eliminated. The whole process is then repeated six or seven times. (d) Then the free end is passed through the loop and the knot tightened down. For subcuticular sutures the knot can then be buried before the thread is cut.

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After tying the knot, the ends are trimmed. These need to be short, so that no excess foreign material is left in the wound, but they should not be cut so short that the knot becomes untied. For sutures that are to be removed, leaving a long end facilitates removal, but they should not be left so long that the animal is encouraged to interfere with them.

Suture patterns

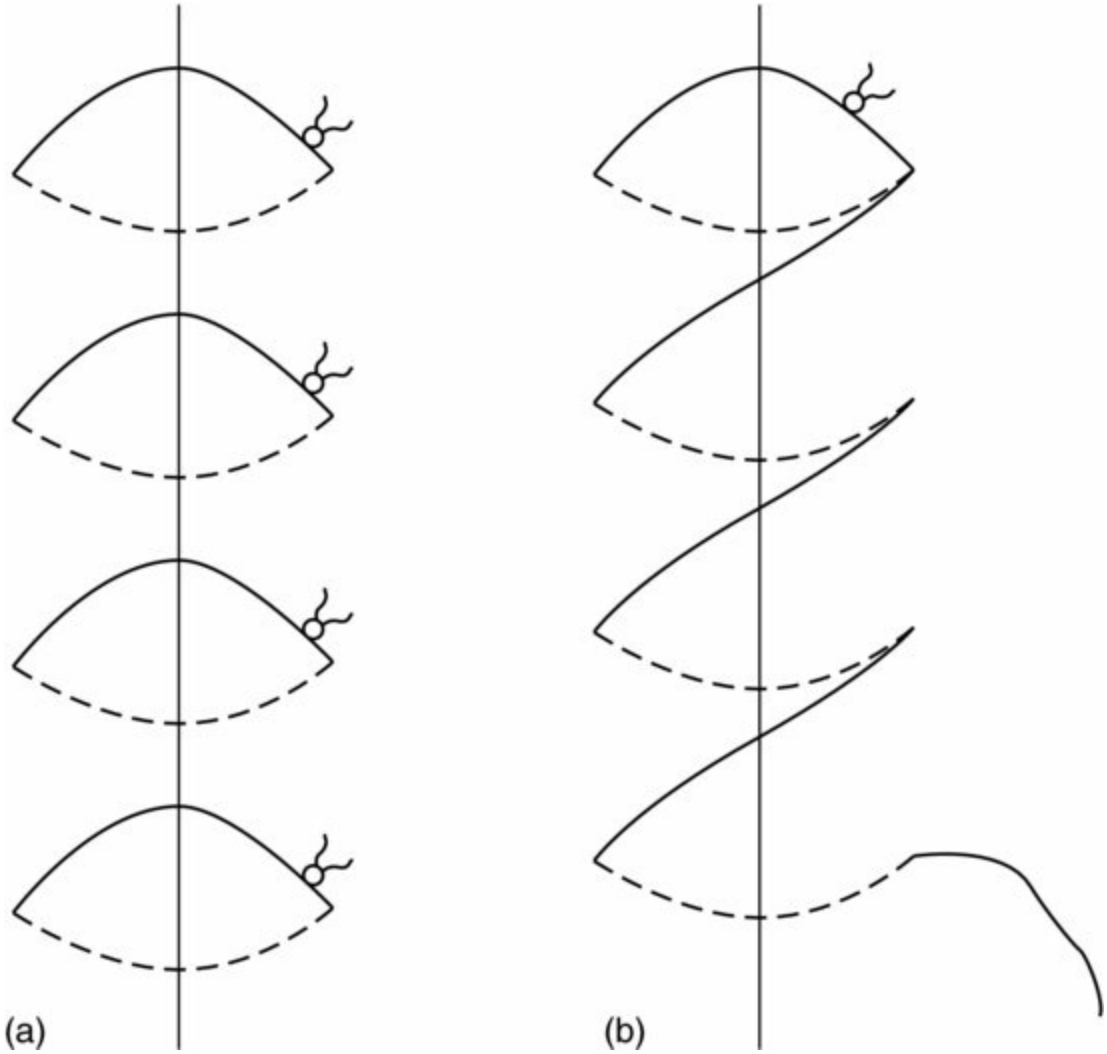
The aim when suturing is to bring the tissue margins together, avoiding excessive tension or pressure on tissues, to optimise healing. Suture patterns may be apposing, where the edges are just brought together, everting, where the tissue edges are turned out, or inverting, where the tissue edges are turned inwards. The pattern chosen depends on the security required, whether a good seal is needed, and whether there is tension on the wound. Apposing patterns generally produce the best result since they bring the tissues together in a more natural configuration, but everting patterns are sometimes used where there is tension on the wound, and inverting patterns are used where a good seal is needed, for example in the gut. There are many specialised suture techniques for particular tissues and specialised surgical texts should be consulted for these, such as Baines et al. (2012)[16] or Fossum (2007)[17].

Suture patterns can be divided into *interrupted* or *continuous* patterns. In interrupted patterns, a knot is tied after each suture is completed, whereas in a continuous pattern the thread is repeatedly passed through the layers of tissue and only knotted at the beginning and end. Obviously, the former is slower but has the advantage of added security: if one suture breaks down, or one knot comes undone, the whole row is not lost. The latter is quicker but less secure.

With a *simple interrupted suture*, the suture material is passed through the tissue on each side of the wound once and then tied, so each stitch is tied separately. This produces good wound security, since if one suture breaks the remaining sutures are unaffected. This knot may be used in the skin or underlying tissue layers, it produces good approximation of wound edges, but it is slow to insert (Figure 10.14a).

FIGURE 10.14 Suture patterns. (a) Simple interrupted suture pattern. (b) Simple continuous suture pattern.

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Simple continuous sutures involve placement of several stitches from one continuous length of suture material, with a knot tied at each end. This is quick to insert, but there is reduced security compared with interrupted sutures. They can be used where security not an issue (Figure 10.14b).

Mattress sutures, where the knot is tied after two passes through the tissues, are quicker to insert than simple interrupted sutures and still give good security.

The *horizontal mattress suture* is quicker to insert than a simple interrupted suture and provides reasonable security, but is less comfortable for the animal (Figure 10.15) because it everts the wound edges, which predisposes to infection and scarring along the suture line. This is sometimes used if there is tension on the wound. Better is the *cruciate suture*, an apposing suture where the suture material is tied in a cross pattern (Figure 10.16b).

FIGURE 10.15 Horizontal mattress suture pattern.

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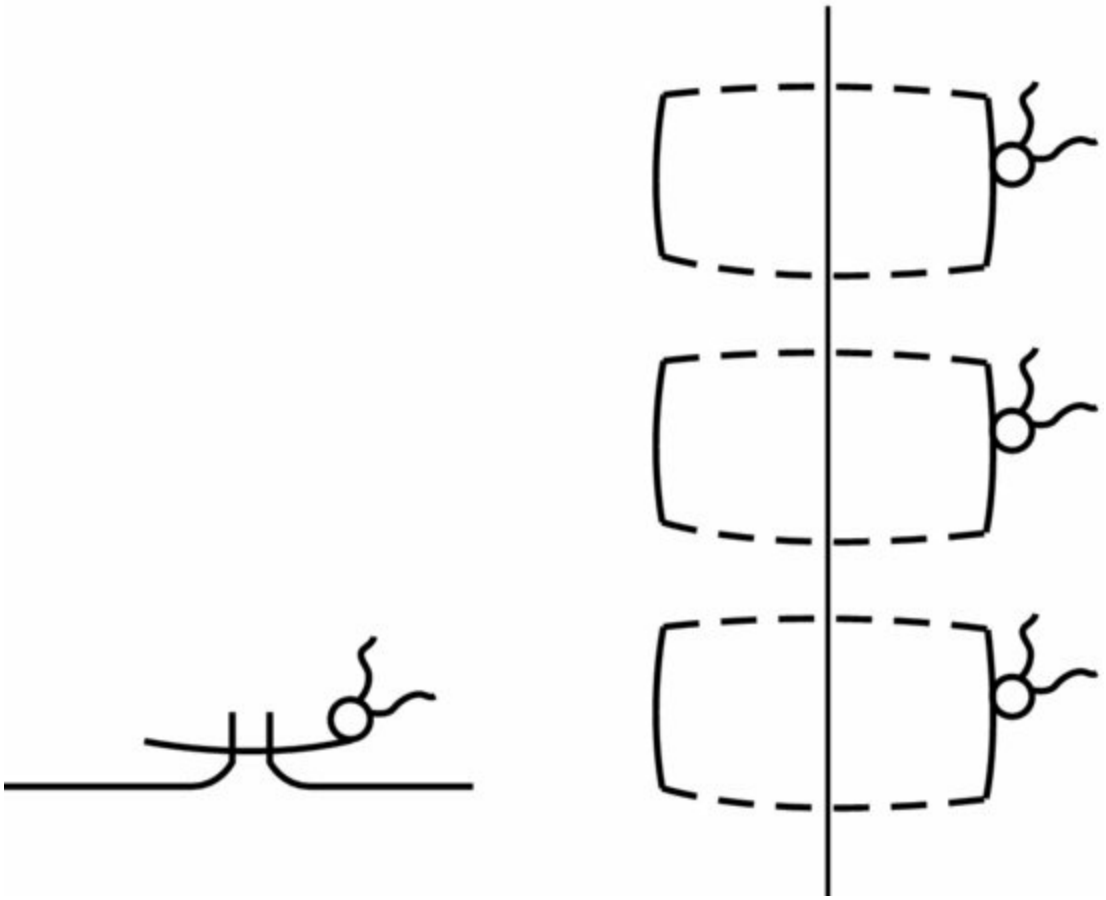
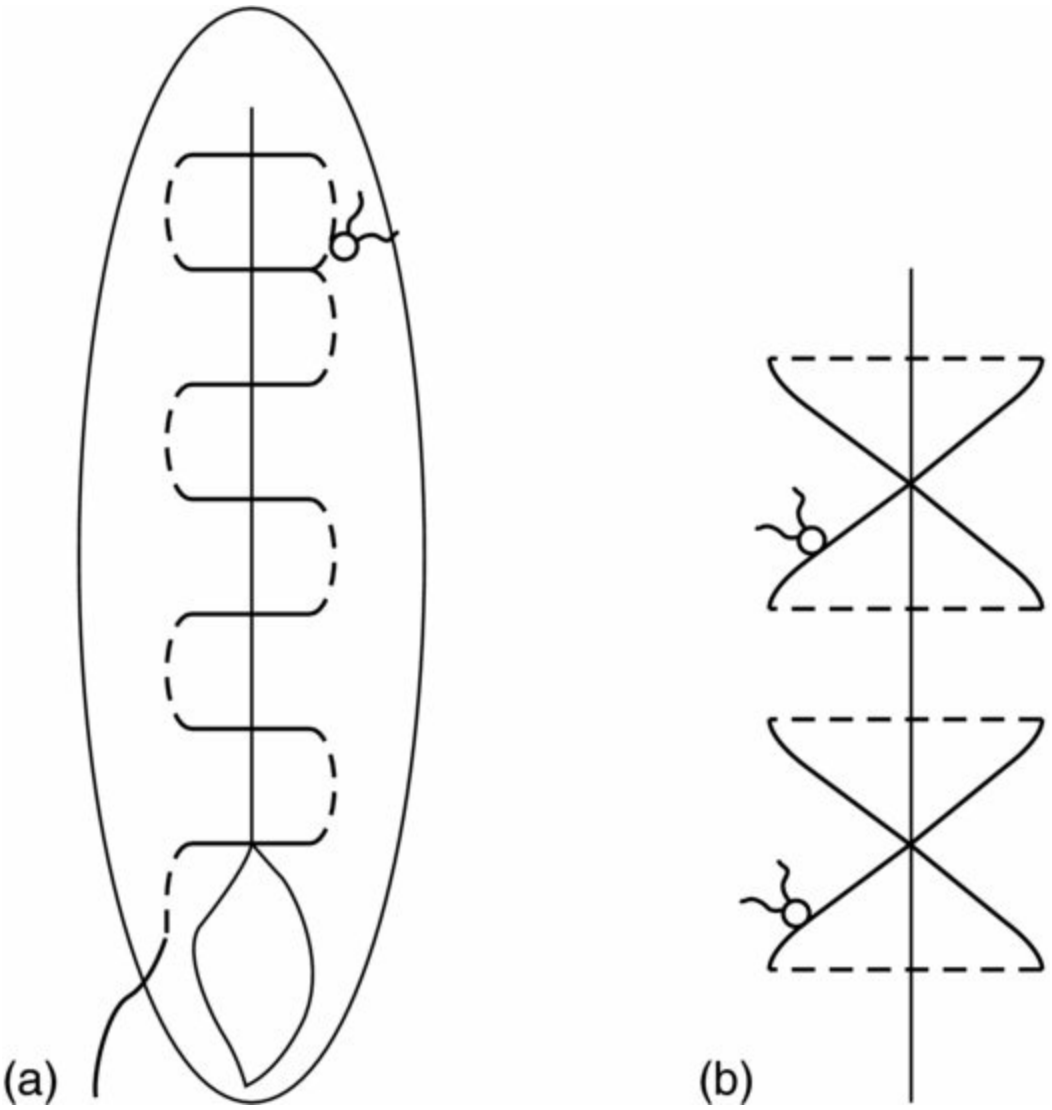


FIGURE 10.16 (a) Subcuticular suture pattern. (b) Cruciate mattress suture pattern.

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A *subcuticular suture* can be used for the skin if it is thick enough. This is essentially a continuous horizontal mattress suture in the subcutis, closing the wound with the suture material being completely buried under skin. This produces good apposition of the wound edges, and no suture material is left on the skin surface, thus reducing chances of interference from the animal (Figure 10.16a).

Suture materials

There is a wide choice of both type and size of suture materials available. The choice of suture material and pattern depends on the nature and location of tissue (buried or in skin), the length of time needed for healing to occur and the consequences of breakdown (security required). The ideal suture material has good handling characteristics and knot strength, does not induce a significant tissue reaction and disappears as soon as healing has occurred. No single suture material has all these characteristics, and no one type is suitable for all situations.

Different tissues have different healing rates, and therefore need suture materials to support them for different periods of time. The peritoneum heals within a few days, skin takes 7–10 days, tendons can take a month or more and fascia only regains 70% of its original strength 9 months post-operatively.

Suture materials may be absorbable or non-absorbable, natural or synthetic, and monofilament (single-stranded) or braided (several thin strands twisted or woven together).

Absorbable suture materials are broken down in the body and will eventually dissolve. They are generally used in buried or

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visceral locations, although can be used in skin if it is not possible to take them out at a later date. Non-absorbable sutures are only used in skin from where they can be removed, or in very specific situations where prolonged support is required (e.g. bones, tendon repair).

Natural suture materials such as catgut and silk have mostly been superseded by superior modern synthetic materials and their use is not generally recommended. Synthetic suture materials have predictable features and are quality controlled, and cause less tissue reaction than natural materials. Some are available impregnated with antibacterial agent, reducing the risk of infection developing along the suture line (e.g. Coated Vicryl Plus and Monocryl Plus, both from Ethicon).

Monofilament sutures are made from a single strand of material. Because they are smooth, there is minimal drag as they are pulled through the tissues and they cause less trauma than braided sutures. Also, braided sutures give rise to post-operative infections more easily than monofilaments if used in the skin. This is because fluids are drawn from the end of the suture into the knot and the tissue by capillary action, leading to micro-abscesses along the suture line. However, monofilament suture are springy and hard to handle, especially thicker gauges, and can be difficult to tie securely as knots tend to slip. Braided sutures are much easier to handle, and give better knot security.

Absorbable sutures

The only natural absorbable suture material in use is *catgut*. This is made from bovine or ovine intestinal connective tissue. It is absorbed unpredictably by the action of cells and enzymes, and actively promotes inflammation. It has been known to disappear in as little as 72 h if implanted in the presence of infection where there is a high white cell count, with resultant wound breakdown. Its use is not recommended.

Many synthetic absorbable suture materials are available with different properties, so an appropriate material can be chosen to suit the tissues being sutured.

Polyglactin 910 is a synthetic absorbable braided suture which is absorbed by simple water hydrolysis and does not promote an inflammatory response. It gives a predictable 21 days' support in tissue. Once its holding strength has gone, it is absorbed completely in 70 days. Examples include Coated Vicryl, Coated Vicryl Plus (both from Ethicon) and Surgicryl 910 (SMI).

Vicryl Rapide is also made from polyglactin but it provides 66% of the initial strength of ordinary Vicryl, and loses its tensile strength more rapidly and it is absorbed in less time. It usually falls off or can be wiped away 7–10 days post-operatively. It provides short-term wound support with greatly increased comfort for the animal and is ideal for closure of skin and mucosa following minor surgery.

Several sutures derived from *polyglycolic acid* are available, with different characteristics. Examples include Surgicryl PGA, Surgicryl Rapid (both SMI), Polysorb, Dexon (braided and monofilament) and Maxon (all Covidien).

Sutures made from *poliglecaprone 25* are absorbed slightly more quickly than polyglactin, also by hydrolysis and also completely predictably. At 7 days post-implantation there is about 55% of the original strength remaining. These sutures are monofilament, and so there is no capillarity and they glide through tissue easily with no drag effect. Although monofilament, they are supple and easy to handle and the knots are quite secure. Examples are Monocryl, Monocryl Plus (both Ethicon) and Surgicryl Monofast (SMI).

Polydioxanone sutures are monofilament sutures with high tensile strength, which provide wound support for more than 42 days. It has reasonable handling and knotting properties. Examples are PDS (Ethicon) and Surgicryl Monofilament (SMI). See Table 10.2.

Table 10.2 Absorbable sutures.

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Material	Tissue reactivity	Knot security	Handling properties	Example	Structure	Effective wound support (days)	Absorption time (days)
Poliglecaprone	Minimal	Fair	Good	Monocryl and Monocryl Plus	Monofilament	7	91–119
				Surgicryl Monofast	Monofilament	7	120
Polyglactin	Minimal	Good	Good	Vicryl and Vicryl Plus	Coated and braided	21	70
				Surgicryl 910	Coated and braided	30	56–70
				Vicryl Rapide	Braided	5	42
Polyglycolic acid and glycolide polymers	Minimal	Fair	Good	Surgicryl PGA	Braided	30	60–90
				Surgicryl Rapid	Braided	10	42
				Polysorb	Coated and braided	21	56–70
				Dexon	Braided or monofilament	14–21	60–90
				Maxon	Monofilament	42	180
				Caprosyn	Monofilament	10	<56
				Biosyn	Monofilament	21	90–110
Polydioxanone	Minimal	Fair	Fair	PDS	Monofilament	42	183–238
				Surgicryl Monofilament	Monofilament	60	180–210
Catgut	Moderate	Fair	Fair	Catgut plain	Twisted	7–10	60–70
				Catgut chromic	Twisted	21–28	90

Non-absorbable sutures

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The main natural non-absorbable suture is *silk*. Silk is braided, and although it has excellent knot security and handling properties, when used in a skin surface location it tends to lead to micro-abscessation along the suture line. Examples include Perma-Hand (Ethicon) and Sofsilk (Covidien).

The classic synthetic non-absorbable material is *nylon* (polyamide). This is available as monofilament or braided. Larger sizes of monofilament nylon can be difficult to tie. The braided form is also available with a sheathed coat, which improves handling and reduces capillarity (e.g. Supramid, SMI). It is not generally used as a buried suture as it can cause problems such as sinus formation. Examples include Ethilon (Ethicon), Daclon (SMI), and Monosof and Dermalon (both Covidien), which are monofilament, and Nurolon (Ethicon) and Surgilon (Covidien), which are braided.

Monofilament *polypropylene* is totally inert and causes no reaction when buried. It handles fairly well**,** and can be used in skin and as a buried suture when prolonged support is needed. It is good for ophthalmic and cardiovascular surgery because it flexes with the action of pulsatile tissue. Examples include Prolene **(**Ethicon), Polypropylene (SMI) and Surgipro (Covidien).

Braided *polyester* can be obtained plain (e.g. Mersilene, Ethicon; Surgidac, Covidien) or coated with a non-reactive compound, which acts as a lubricant, improving the handling properties of the suture; for example, Ethibond Excel (Ethicon), Ti-Cron (Covidien) and Polyester (SMI).

Stainless steel is available as wire, staples and wound clips. Staples are supplied pre-sterilised inside a special applicator. Staples are widely used in human and veterinary surgery, and applicators are available for specialised surgical techniques which can deliver a row of staples in one go. Skin staples are inserted individually, and the main advantages of staples are their speed and ease of application and that they allow room for the normal post-operative swelling that occurs in the first phase of wound healing. However, most skin staples are too big for use in small laboratory animals. Staples are removed after 10 days with a purpose-made device. Removal can cause mild pain and may be resented by some animals.

Surgical stainless steel suture is available as a monofilament, indicated for use in abdominal wound closure, hernia repair, sternal closure and orthopaedic procedures including cerclage and tendon repair. Examples include Steel Monofilament (SMI) and Steel (Covidien).

Wound clips are applied using special applicator forceps and have to be sterilised before use. They have a tendency to evert wound edges, which leads to increased scarring and interference from the animal. However, they are quick to insert and are useful when closing a small skin wound. Different types of wound clips are available. *Autoclips* (Harvard Apparatus) come with a special applicator which is easy to load, sterilise and use, and the applicator prevents overtightening of the clips. These are also easy to remove, using a specially designed remover. *Michel clips* have to be sterilised and loaded individually into forceps for application and then tightened using digital pressure. There is a tendency for them to be overcrimped, leading to tissue damage and necrosis which acts as a focus for wound infection. They are also difficult to remove and their use is not recommended. See Table 10.3.

Table 10.3 Non-absorbable sutures.

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Material	Tissue reactivity	Knot security	Handling properties	Example	Structure	Effective wound support
Polyester	Moderate	Poor	Good	Ethibond, Ti-Cron, Polyester	Braided and coated	Permanent
				Mersilene, Surgidac	Braided	
Stainless steel	None	Excellent	Poor	Surgical steel suture, wound clips and staples	Monofilament	Permanent
Polypropylene	Minimal	Good	Fair	Prolene, Surgipro	Monofilament	Permanent
Nylon	Minimal	Poor	Poor	Ethilon, Monosof, Dermalon	Monofilament	Gradual loss
		Fair	Good	Nurolon, Surgilon	Braided	
		Fair	Good	Supramid	Braided and sheathed	
Silk	Severe	Good	Excellent	Perma-Hand, Sofsilk	Braided	Gradual loss

Tissue adhesives

For small skin wounds, where there is no tension and when there is unlikely to be interference from the animal, wounds may be

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closed using *n-butyl cyanoacrylate* adhesive (Vetbond, 3M; Dermafuse, Millpledge). On contact with tissue and body fluids it changes from a liquid to a solid state by rapid polymerisation and thus seals the wound. It is also useful as a supplement to sutures or staples. It should only be placed *between* the sutures or staples and not on them, or it may interfere with removal.

Sizes of suture

Sutures come in a variety of thicknesses. In the metric system, the diameter is represented in tenths of a millimetre. In the United States Pharmacopeia (USP) system the average suture is designated size 0, and as thickness decreases 0s are added (2-0, 3-0, 4-0, etc., where the larger the number the thinner the suture material). Thicker sizes for use in large animals are denoted by numbers (1, 2, etc.). For rodent work, a suture material thickness of 1.5 metric (4-0 USP) or 1 metric (5-0 USP) or smaller is likely to be appropriate. The thickness of the suture required depends on the nature of the tissues and the size of the animal. Choose a thickness of suture material adequate to provide sufficient tensile strength to hold the tissues together, while minimising the amount of foreign material in the wound, remembering that using too thick a suture material will impair healing. Synthetic absorbable materials are much stronger than natural materials, so a thinner gauge can be used.

Packaging

Sutures are supplied both individually packed in different precut lengths or on reels to be cut by the user, and may be coloured to enhance visibility of the suture. Modern sutures are supplied pre-sterilised in a double-layered pack. The pack can be opened by a non-sterile assistant, revealing the sterile inner pack. Suture packs will display an expiry date: after this date absorbable sutures begin to lose their strength and the time for absorption becomes unpredictable. It is unwise to use out-of-date sutures and risk wound breakdown.

Example: closure of a midline abdominal incision (laparotomy) and enterotomy in a rat

In closing such an incision, typically four layers of tissues may need to be closed as follows: gut wall, muscle, fat and skin.

- *Gut wall*: it is important to make a good seal to avoid leakage of gut contents. The gut heals very quickly so prolonged suture strength is not required, and there is no tension on the wound so breakdown is unlikely. The gut wall should be closed with a fine-gauge, absorbable monofilament suture such as Monocryl Plus, using a round-bodied needle. A continuous apposing or inverting suture pattern would be appropriate.
- *Muscle layer*: the actual holding power of muscle is poor: the layers that surround it are much stronger (fascia and peritoneum in this case), so when placing sutures in the abdominal body wall ensure that the fascia and peritoneum are both included in the suture. This should be closed using a fine-gauge, absorbable, braided suture material such as Vicryl or Polysorb, using a cutting needle, and an apposing interrupted suture pattern to ensure good knot security and security of the suture line.
- *Fat layer*: simple interrupted sutures could also be used here, but in practice a simple continuous pattern using an absorbable braided suture is often used for speed.
- *Skin*: if the skin is thick enough it can be closed with a buried, subcuticular suture in a braided absorbable material. Otherwise, a simple interrupted or cruciate suture pattern will give the best security, using a monofilament absorbable or non-absorbable suture material with a cutting or reverse cutting needle.

Summary

When performing experimental surgical procedures, you should aim to:

- achieve asepsis and avoid post-operative infections,
- minimise inflammation by using good techniques and appropriate instruments,
- minimise haemorrhage by careful surgery and meticulous haemostasis,
- produce good wound apposition by using suitable suture materials and patterns,
- provide good post-operative care and minimise recovery time.

These can be achieved with a little preparation and practice. Following the principles described above will result in improved science and better experimental data, as well as contributing to improved animal welfare. Your designated veterinary surgeon will

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be able to advise and guide you.

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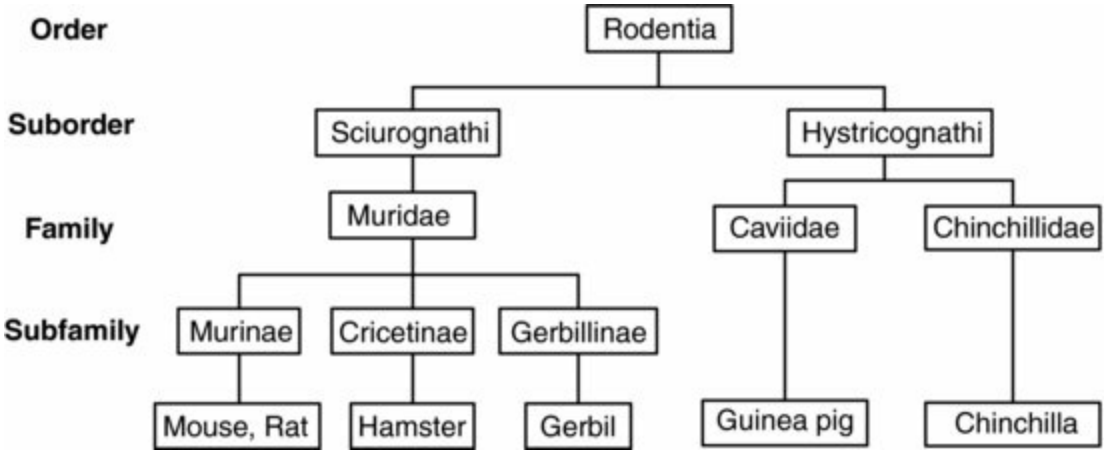
Chapter 11

Small Laboratory Animals

Rodents

Rodents are a very successful order of mammals. They are found colonising almost all habitats, there are currently 2277 different species[1], and new species are being discovered continually. As new techniques for genetic and phenotypic evaluation are developed, the classification of rodents changes, making this a dynamic and changing group[2]. Some studies have suggested that guinea pigs should be classed in a separate order, based on amino acid sequences[3]. More recent studies using wider sampling methods have restored guinea pigs to the order[4,5], but within the order no single classification system has yet been universally adopted. Most classification systems divide rodents into the suborders Sciurognathi or Hystricognathi according to the shape of the lower jaw. One such classification system is illustrated in Figure 11.1[6,7].

FIGURE 11.1 Classification of rodents.



Rodents accounted for 81% of animals used in research in the UK in 2010[8], with mice comprising over 72% of animals used. Mice are used in studies including genetics, oncology, toxicology, immunology and developmental biology. Mice are used because they are small, have a high fertility rate and short gestation period, and are easy to maintain. They are also susceptible to many genetic diseases that afflict humans. Rats are used in studies including nutritional research and neuroscience.

Rodents have a number of distinguishing features. Despite their success, all members of the order are small: the largest rodent, the Capybara, weighs only 30–50 kg and is about the size of a small pig.

Dentition

The word rodent derives from Latin, meaning ‘to gnaw’. This apt description relates to the extremely sharp, chisel-like incisor teeth. Rodents have a single pair of incisors in upper and lower jaws. These are open-rooted, and grow throughout life. For example, an adult rat's upper incisors may grow 2.2 mm each week, (0.31–0.32 mm per day), and the lower incisors about 2.8 mm per week (0.4 mm per day)[9]. The teeth are worn away by chewing, and by bruxing (tooth grinding). The incisor teeth have enamel only on the labial (outer) surface, with softer dentine on the inner surface which wears away more quickly, ensuring the teeth always have a sharp edge. The jaw can move both sideways and forwards and backwards, to facilitate gnawing and bruxing.

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Nutrition and digestion

Rodents have adapted to feed on a variety of foodstuffs, including seeds and grains, roots, leaves or fruits, as well as invertebrates and even small fish. Laboratory species eat mostly vegetable matter and cereals. Rodents have a simple stomach, with glandular and non-glandular parts, and cannot vomit. A fold of muscular tissue at the gastro-oesophageal junction forms a physical barrier, and they don't have the oesophageal muscle strength to overcome this barrier. Also they appear to lack the ability to coordinate the different muscles required for vomiting. Rodents, like other herbivores, require a microbial fermentation chamber in which digestion of cellulose from plant material can occur. This takes place in the caecum, located between the ileum and the colon. However, since this is at the end of the digestive tract, the digested matter has to be re-ingested in order for nutrients to be absorbed. During the dark period, pellets of digested material from the caecum are coated with mucus, and these caecotrophs are ingested directly from the anus, allowing the nutrients to be absorbed. This is known as coprophagy. Undigested material that has been through the digestive tract twice is expelled as dry pellets, which can be seen in the environment.

Rodents in the laboratory are usually fed *ad libitum* with a complete cereal-based pelleted diet, from hoppers suspended above the floor to prevent faecal contamination. These should be cleaned weekly, but the cleaning routine will depend on factors, such as the housing system, stocking density, or presence of newborn litters. Disease states and pregnancy affect the food requirements.

Water

Water is required for lubrication of the food as well as hydration, particularly in mice, and if insufficient fluid is available mice have difficulty eating. It may be supplied in automated systems or bottles. Water provided in bottles becomes contaminated by saliva and faecal matter, and frequent cleaning is required. For some strains, acidification or chlorination of the water may be needed to prevent disease arising in the animals from contaminated water. Automated systems ensure that there is a constant supply available, but when used with solid-bottomed cages there can be a risk of flooding, as animals may play with the sipper tubes or push bedding material into the tube, causing it to drip. When ill, mice drink very little and rapidly dehydrate. Medicines administered in the water may be ineffective, and care must be taken to ensure that adequate quantities of fluid are administered by other means.

Behaviour

Rodents are mostly social animals, living in groups and defending territories from intruders. A considerable amount of rodent behaviour is directed towards marking and maintaining their territory. Most rodents are crepuscular (active at dawn and dusk), or nocturnal. They often exhibit burrowing behaviour if presented with a suitable substrate, and this can be used to assess welfare[10].

Senses and communication

Scent marking is particularly important in rodents. It helps maintain a familiar and secure environment, attracts mates and repels intruders, and helps the animal orientate itself. Removal of scent marks, such as occurs during cage cleaning, can lead to insecurity and increased aggression and leads to increased marking. Animals often form a dominance hierarchy, which helps to regulate conflict and reduce aggression and injury. However, fighting can and does occur, particularly between similarly matched individuals. If the territory comes under threat, rodents will mark and defend the territory, fighting if necessary. If an animal perceives the scent of an intruder but cannot then identify them, this can lead to stress.

Rodents have little or no colour vision, being nocturnal. They hear in the ultrasound range, mice detecting frequencies up 100 kHz (compared to about 20 kHz in humans), and are most sensitive in the 15–20 kHz range and around 50 kHz. They communicate both in the human audible range with squeaks (for long-distance warnings), and in the ultrasound range (for short-distance communication).

Housing

Rodents can be housed in conventional units where the pathogen status may be unknown, but more frequently are kept in barrier units with a defined and monitored health status. Cages are often of the shoebox type made of plastic. Rodent housing should have solid floors, with environmental enrichment, nesting and bedding material, and refuges, allowing them to exhibit a range of natural behaviours. Most species are sociable and can be housed in groups of compatible animals, although adult male mice may fight, and are sometimes housed alone, and hamsters are solitary by nature. Females of all species with litters will defend their

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young and are best separated while nursing.

Environment

To comply with the requirements of EU Directive 2010/63[11], rodents require temperatures between 20 and 24°C, humidity between 45 and 65% and 12 h of daylight daily. The light intensity should be 350–400 lux, except for albino animals. For these, it should be less than 60 lux to avoid damage to the retina. Rodents also hear in the ultrasound range (the wild mouse has an upper frequency limit of 92 kHz)[12], and care should be taken to monitor and reduce ultrasound in facilities, since many pieces of equipment and everyday activities such as running a tap may produce ultrasound.

Anaesthesia

Anaesthesia in small laboratory animals is associated with significant risk. Their small size makes access to blood vessels difficult and increases heat loss during anaesthesia, resulting in hypothermia. Anaesthesia should only be induced in healthy, stress free animals. Small animals need to be kept warm during and after anaesthesia, to prevent hypothermia, and may need supplementary fluids to prevent dehydration and encourage return to normal physiology in the post-anaesthetic period.

Anaesthesia can be induced by inhalation or injection, and inhalation is the preferred method for small-animal anaesthesia. It has a very high safety margin for all of the laboratory species and is rapidly excreted from the tissues via the lungs, which results in rapid recovery. There is virtually no biotransformation, making it the most suitable agent for studies that require maintenance of normal drug-metabolising ability.

Many anaesthetic agents are available (see Chapter 9). Drug doses are intended only as a guide and will have to be adjusted to take account of varying responses of different animals and strains.

Mouse

The mouse, *Mus musculus*, is the most commonly used laboratory animal. Many well-defined inbred strains and outbred stocks are available, for which the karyotypes are known. More is known about the genome of the mouse than any other species. An international project has undertaken to sequence the entire mouse genome, and there are numerous centres working on producing genetically modified mice that model human diseases. There are many types of genetically modified mouse available that are useful models for specific disease entities (see Chapter 6).

Behaviour

Mice are active, social animals, which naturally live in harmony in groups with one male and several females and their young once their hierarchy is established. They live in a variety of places near food sources, and construct nests from various soft materials. They exhibit a wide range of behaviours including exploring, foraging, climbing, grooming and resting. Mice usually run on all fours, but when eating, fighting or exploring they stand on their hind legs, supported by the tail. Mice are good jumpers, climbers and swimmers. Opportunities to perform as many of these behaviours as possible should be provided in the laboratory. Failure to provide an adequate variety of enrichment may lead to stress and abnormal behaviour, confounding experimental results.

Mice are territorial, and dominant males respect each other's territory, venturing into another's territory only if it is vacant. If two or more males are held together in a cage they will often show aggression unless they have been raised together from birth.

Mice are afraid of rats, which may kill and eat mice, so these species should not be held in the same air space.

Communication

Mice use pheromones to communicate, and it is vital to understand this communication in order to manage these animals successfully in the laboratory. Some of the effects of pheromones are listed below:

- stress in one mouse causes dispersal of other mice,
- female mice attract male mice, and vice versa,
- pheromones from lactating females attract the young,
- foreign females stimulate aggression by other females,

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- foreign males provoke aggression from other male mice, and may cause recently mated females to abort (Bruce effect),
- group-housed females become anoestrus, and resume cycling if a male is introduced (Whitten effect),
- co-existing males emit pheromones to reduce aggression within the group, but which cause foreign males to avoid the territory.

Communication in mice through scent marking has been extensively studied. Two types of information are carried in scent marks: genetic information (fixed), and information on current status (variable; depending on diet etc.). Scents are detected by the main olfactory system, and via the vomeronasal organ, which detects involatile compounds. This is very finely tuned to specific compounds such as pheromomes.

Scents are composed of two components: airborne volatile compounds and small peptides, and major urinary proteins (MUPs), which are specialised communication proteins. Most MUPS are excreted in urine, but some are also excreted by other routes. MUPs in scent marks signal the genetic identity of the mouse to other mice, for example their sex and whether they are a relative. Individuals have different proteins, allowing animals to recognize each other and avoid mating with their relatives[13].

Airborne volatiles allow the scent source to be identified from a distance. If the scent is known, the animal will tend to ignore it. If not, they will try to identify the source of the scent and investigate the MUP pattern, to determine the exact identity of the scent marker; that is, *remote* sensing via the airborne volatile compounds leads to *contact* sensing of the MUP pattern. The mouse then learns an association between the airborne volatile scent and the MUP pattern. The next time it encounters the airborne volatile it will be recognised and the MUP may not need to be investigated[14].

Female mice can distinguish between dominant and weaker males by how fresh and dense a male scent mark is. Dominant individuals mark more than others, so leave more dense markings, and they also leave the most recent scent marks. Subordinate males scent mark to a lesser extent. Females are attracted to the airborne scent of familiar males and choose males with the most recent scent marks[15].

When mice meet, the dominant male allows the intruder to sniff him before he attacks; this gives the intruder a chance to recognise him and run away, thus avoiding aggressive encounters.

Implications for laboratory mice

All laboratory mice have been shown by mitochondrial DNA to have come from a single female, and they therefore have very little variation in their MUP[16]. Thus they share common genetic signals: this reduces aggression between individuals. However, animals also recognise each other through non-genetic factors influencing the airborne volatiles, and changes in these airborne volatile signals can lead to aggression. Some experimental treatments may influence the airborne volatile signals, and can therefore increase aggressive encounters.

If mice are only exposed to airborne scents without finding the source for contact sensing, mice will eventually ignore it (this happens on racks of conventional cages). However, if they are then exposed to the contact scent of unfamiliar animals, this may lead to an aggressive response. Thus placing unfamiliar animals into shared equipment without cleaning between them can lead to aggressive behaviour and stress. Pheromones are used to maintain stability in the colony and if they are removed each time the cage is cleaned fighting will ensue and subordinate mice will be barbered (where the dominant mouse chews the whiskers of subordinates), or possibly injured (Figure 11.2).

FIGURE 11.2 Barbering in the mouse.

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Keeping mice in compatible groups, providing environmental enrichment such as tubes, objects to climb on and refuges, and leaving a little of the soiled bedding in the new cage to reduce the need to keep re-establishing the dominance hierarchy and territory marking all help to reduce aggression and stress.

There are strain differences in behaviour. For example, male BALB/c mice are particularly aggressive, and fight wounds are common.

Feeding

Estimating the quantitative nutrient requirements for mice is difficult, since different strains have different requirements, and there is little published information on nutrient requirements for maintenance and production in mice[17]. Different environments also affect nutritional requirements: animals from conventional, specific-pathogen-free or germ-free environments have different intestinal flora, and these influence nutrient requirements. Temperature, diet composition, physiological status, microbiological status and genetic background influence maintenance energy requirements: obese mice have a lower energy requirement than lean mice, and females a lower requirement than males. The daily metabolisable energy (or ME) requirement for maintenance is approximately 160 kcal (670 kJ)/$BW_{kg}^{0.75}$/day in mice with no genetic or stress-induced abnormalities17. Diets for laboratory mice may be pelleted (compressed or expanded), powdered or in gel form. Diets for maintenance typically contain 4–5% lipid and 12–14% protein, and diets for growth and reproduction contain 7–11% lipid and 17–19% protein[18]. Mice will eat approximately 3.5–6.2 g of pelleted diet daily depending on strain and physiological status17.

Environment

Mice have a large surface area to volume ratio and lose heat rapidly, and so are sensitive to changes in ambient temperature. Much energy is expended in maintaining body temperature, and they cannot tolerate a reduction in room temperature. Mice are also susceptible to water loss. They cannot afford to sweat or pant to lose heat, as this would cause dehydration. In the wild they use behavioural mechanisms, such as burrowing, to keep cool. Therefore, maintenance of the correct environmental conditions and provision of bedding and shelters to allow the animals to manipulate their own microenvironment is vital.

Breeding

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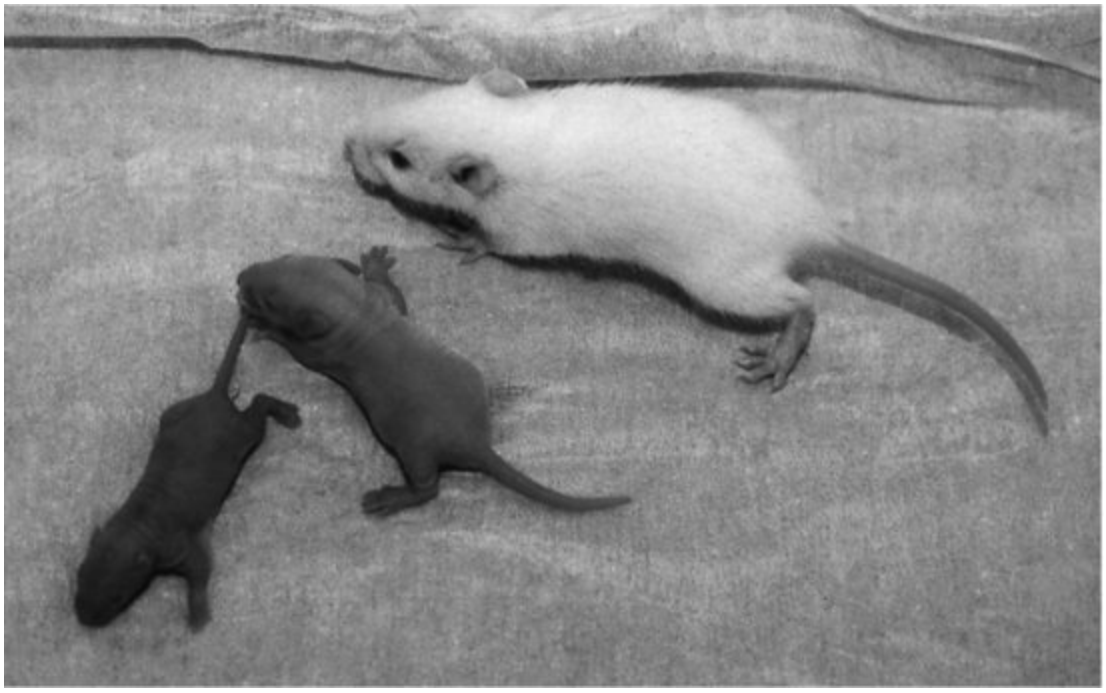
Male mice reach puberty at approximately 7 weeks, and females at 6 weeks. Females then cycle every 4–5 days. This is affected by photoperiod and the presence of others, a period of 12–14 h light daily is needed to maintain oestrous cycles18. Oestrus, mating and ovulation tend to occur during the dark phase of the light cycle. The Whitten effect can be used for synchronising oestrus or for timed matings. Mating results in the formation of a vaginal plug, which can be detected to confirm mating. Fetuses can be palpated from day 7–10. Gestation lasts 19–21 days and 1–15 pups may be born, depending on the strain. Females have a post-partum oestrus within 24 h of giving birth, and if kept with the male will be mated again at this time.

Mice may be bred using a harem system, with one male for two to six females, pregnant females being removed from the group to give birth, or may be kept together in a monogamous system. With the latter system, the young are removed before the next litter is born. Mice will breed until they are 12–18 months old, although the economic breeding life of most strains is around 6 months.

Growth

Mouse pups are dark purplish red at birth, and may have a visible milk spot in the abdomen. Their eyes and ears are closed and there is no hair coat. They become lighter in colour over the first few days, and in coloured mice pigment may be visible by day 3. By day 4 the ear flaps are starting to separate from the head, and this is complete by day 5. Hair appears from day 7 and covers them fully by day 10. From day 11, teeth begin to erupt and the eyes begin to open. These are fully open by day 12, and they start nibbling solid food[19] (see Figure 11.3). They become increasingly active and increase their intake of solid food until weaning takes place at about 21 days. Weaning should only be carried out if the animals are large enough, and for slower growing strains this may take up to 28 days. Strains vary dramatically in their rate of growth, with some strains growing twice as fast as others17. In general, outbred stocks grow much faster than inbred strains.

FIGURE 11.3 Ageing the rodent. (From left to right.) At 4 days old, the pup is blind and deaf with no hair. At 8 days old, the pup is still blind and deaf but is covered with fine hair. At 18 days old, the eyes and ears have opened, the hair coat is well developed and the animal is increasingly independent.



Handling

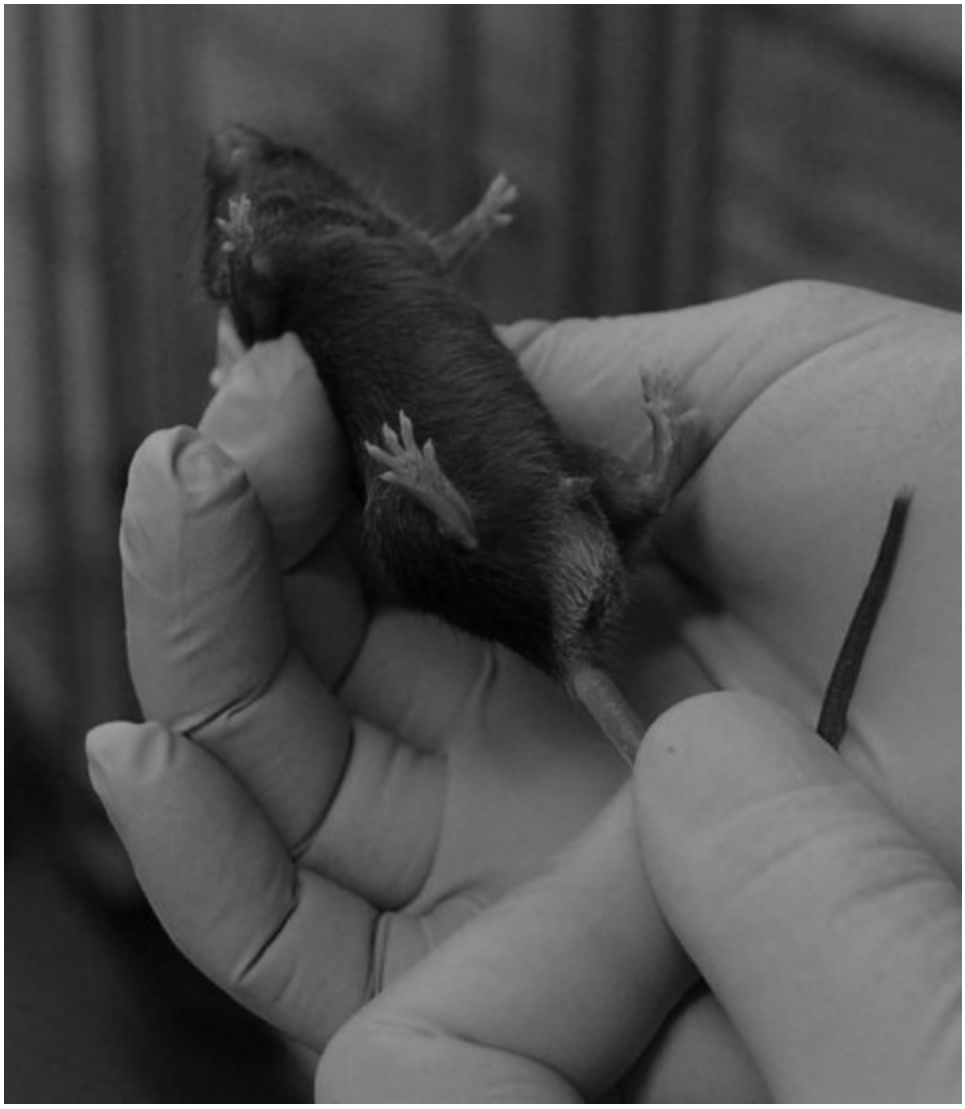
Mice are generally easy to restrain, but their small size makes them vulnerable to physical injury. Some mice are also very active and may attempt to jump away from the handler. Movements must be quick and decisive. Handling mice for procedures is potentially a significant source of stress. The most common method used to capture and handle laboratory mice is to pick up and restrain the mouse by its tail; however, this induces a high level of anxiety, and mice do not habituate to handling by this method[20].

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Picking mice up by either placing a tunnel in the home cage for the animal to crawl into or by cupping in the open hands causes less stress, and these methods should be used where possible. Approach the animal after first removing the lid of the cage. Otherwise, they may hide beneath the food hopper, which makes them harder to catch and also increases the risk of being bitten.

If it is necessary to restrain the animal for injection, grasp the base of the tail gently but firmly and lift the mouse. Place the mouse down on a non-slip surface, such as the top of the cage, without releasing the tail. The animal may then be sexed by lifting the tail to expose the perineum. The ano-genital distance in the male is approximately twice that seen in the female. Pull back slightly so the mouse grips the surface. Slide the thumb and index finger of the other hand up the animal's body and grasp the scruff of the neck to restrain the head. The animal is then secure and may be examined or injected safely. Extra restraint may be achieved by holding the tail with the fourth and fifth fingers (see Figure 11.4).

FIGURE 11.4 Restraining the mouse for injection. Photo: James and Steve.



To handle newborn mice, transfer the mother to a separate cage first, to prevent aggression from her. The pups can then be gently rolled into the palm of the hand. To avoid subsequent cannibalism by the mother rub the hands in soiled bedding material to acquire pheromones before handling the pups, and rub the young with nest material after handling before replacing the mother. From 10 days of age they can be handled as adults, but the mother should still be removed first.

Pain and stress recognition

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Familiarity with normal appearance and behaviour is required in order to detect when all is not well. There will be normal variation between healthy animals, due to strain differences, age, diet or cyclical changes in the female due to oestrus, etc. Signs of normal appearance and behaviour include:

- alert, inquisitive and interacting with others in the group,
- well groomed with a glossy coat,
- relaxed with a normal gait: no lameness or ataxia, and not hunched or prone,
- bright and clear eyes,
- normal respiration, not shallow and fast, or deep and laboured,
- colour of the extremities (pink, pale or deep red),
- signs of eating and drinking,
- normal faeces and urine,
- teeth not overgrown and no malocclusion,
- moderate degree of body fat over the backbone,
- normal breeding performance with low neonatal mortality.

Signs of pain and distress include:

- increased sleep time,
- weight loss,
- piloerection and hunched appearance,
- isolated from the rest of the group,
- squeals on handling or pressure on affected area,
- may become more docile (or sometimes more aggressive),
- may eat bedding or neonates,
- abdominal writhing.

Common diseases and health monitoring

Conventionally housed mice may be carrying a number of commensal and potentially pathogenic organisms, and barrier-reared animals can develop infections if there is a breakdown in the barrier. Many infections do not produce clinical signs in adult animals. However, they may cause disease in immunocompromised animals, or interfere with research data. Respiratory infections increase the risks associated with anaesthesia and surgery. Regular health screens should be performed to ascertain the health status or the colony, and to ensure that there are no potentially problematic organisms in the colony (see Chapter 5). Table 11.1 lists some of the pathogens to be included in health-screening protocols. This list is not exhaustive, and more information can be found in reference 21.

Table 11.1 Agents to be included in health-screening protocols for mice (FELASA).

Type of infectious agent	Examples
Zoonoses	Hantaan virus, lymphocytic choriomeningitis (LCM) virus, leptospirosis
Disease-causing agents	Sendai virus, pneumonia virus of mice, *Pasteurella pneumotropica, Staphylococcus aureus*, mouse hepatitis virus (MHV), mouse norovirus, mouse rotavirus (epidemic diarrhoea of infant mice EDIM), Theiler's murine encephalomyelitis virus (TMEV), *Clostridium piliformis* (Tyzzer's disease), *Mycoplasma* spp., endoparasites (e.g. *Syphacia obvelata, Aspicularis tetraptera, Tritrichomonas* and coccidia) and ectoparasites (e.g. *Myobia musculi* and *Mycoptes musculinus*)
Diseases affecting research	Minute virus of mice (MVM), lactate dehydrogenase elevating virus (LDHV), *Helicobacter* spp.

Biological data and useful reference data

Biological data and useful reference data are given in Table 11.2.

Table 11.2 Useful data: mouse.

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Biological data		**Breeding data**	
Adult weight (g)	Male 20–40 Female 18–40	Puberty (days)	28–49, average 42
Diploid number	40	Age to breed male (days)	70
Food intake	15 g/100 g body weight	Age to breed female (days)	60–84
Water intake	15 ml/100 g body weight	Gestation (days)	19–21
Natural lifespan (years)	1.5–3	Litter size	4–15
Rectal temperature (°C)	38–39	Birth weight (g)	1–1.5
Heart rate/min	310–840	Weaning age (days)	18–21*
Blood pressure (mmHg)		Oestrous cycle (days)	4–5
Systole	133–160	Post-partum oestrus	Fertile
Diastole	90–110	**Biochemical data**	
Blood volume (ml/kg)	60–75	Serum protein (g/dl)	3.5–7.2
Respiratory rate/min	60–220	Albumin (g/dl)	2.5–4.8
Tidal volume (ml)	0.18	Globulin (g/dl)	0.6
Haematological data		Glucose (mg/dl)	62–175
Red blood cells ($\times 10^6$/mm^3)	7–12.5	Blood urea nitrogen (mg/dl)	12–28
PCV (%)	39–49	Total bilirubin (mg/dl)	0.1–0.9
Hb (g/dl)	10.2–16.6	Creatinine (mg/dl)	0.3–1
White blood cells ($\times 10^3$/mm^3)	6–15	Cholesterol (mg/dl)	26–82
Neutrophils (%)	10–40		
Lymphocytes (%)	55–95		
Eosinophils (%)	0–4		
Monocytes (%)	0.1–3.5		
Basophils (%)	0–0.3		
Platelets ($\times 10^3$/mm^3)	160–410		

Hb, haemoglobin; PCV, packed cell volume.
*Date of weaning may need adjustment depending on weight of the animal. (Note: There are strain variations especially in genetically modified mice.)

Rat

Rats account for approximately 8% of animals used in research, and 293 905 rats were used in 2010 in the UK8. Rats used in research are mainly derived from the brown or Norwegian rat, *Rattus norvegicus*. Outbred and inbred strains are available. Commonly used outbred strains include the albino Wistar and Sprague–Dawley varieties, and the hooded Lister and Long–Evans strains. Inbred strains include the Lewis, Dark Agouti (DA) and Brown Norway strains. Some transgenic rat strains are also available.

Behaviour

Rats are social animals. In the wild, rats are territorial, and live in social groups in burrows. They usually live in groups of one dominant individual with several subordinates, and they establish and maintain their dominance hierarchies through agonistic behaviours, such as chasing and neck grooming. Usually the subordinate rat flees, and fighting is rare. Rats are usually friendly and easy to handle, although there are strain differences, and males tend to be friendlier than females. They become friendlier with more frequent handling. Rats are nocturnal or crepuscular, being active in the dark period. Their eyesight is poor, and blind rats behave as if perfectly normal. See www.ratlife.org for details of rat behaviour.

Housing

Rats are curious and intelligent creatures and need an enriched environment. They should be group housed in stable social groups,

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with solid floors and nesting material to allow them to create an appropriate microenvironment11. Tubes, houses, shelves or other structures allow the animals to divide their environment and make use of the three dimensional space in the cage. Paper-based commercial bedding, wood shavings or corn cobs may be used. Rats like to stand erect, and so cages with high lids are required. The minimum cage height specified in EU Directive 2010/63 is 18 cm, although the UK standard has been 20 cm and this is preferred.

Feeding

The rat is omnivorous, eating a wide variety of seeds, grains and other plant matter as well as invertebrates and small vertebrates22. The digestive tract is similar to other omnivorous rodents, and coprophagy occurs. Rats do not have a gall bladder. Nutritional requirements are better understood than for many laboratory rodents but still vary considerably with physiological status and developmental stage, strain and sex17. They can be fed *ad libitum* on a complete pelleted rodent diet, containing 20–27% protein and 5% fat. Higher protein levels than this may reduce reproduction efficiency. Rats are cautious eaters and will reject strange food. Rats will eat 5–10 g of feed per 100 g of body weight daily. This equates to 15 g/day for maintenance in young and adult rats, 15–20 g/day in pregnancy and 30–40 g in lactation. As rats age, it may be beneficial to restrict their food intake to 80% of *ad libitum* intake: this increases their life span and reduces the incidence of some types of neoplasia[23].

Water

Water may be provided by sipper tubes or by automated watering systems. The water may need to be acidified or chlorinated to reduce contamination, particularly for immunocompromised rats. Rats drink approximately 5–10 ml of water per 100 g of body weight daily.

Environment

Rats should be kept between 20 and 24°C. Young rats have much brown fat to assist in thermogenesis, and this reduces with age. The humidity should be 45–65%. Rats are less sensitive to temperature changes than mice, but low humidity can lead to ringtail, in which an annular lesion appears around the tail, potentially leading to necrosis and sloughing of the tail. A 12 h light period is adequate for rats but bright light is deleterious, particularly for albino rats, and results in retinal degeneration. The level should be less than 400 lux, or 100 lux for albinos. Photoperiod affects the oestrous cycle, and 12–16 h light is best for optimal breeding. Ventilation is particularly important for rats, as many of their pathogens are aerosol-borne11.

Breeding

Rats become sexually mature at about 2 months, females maturing slightly earlier than males. Breeding is usually carried out from 3 months, when females weigh 250 g and males 300 g, and rats breed until they are 12–18 months old. Oestrus occurs every 4–5 days. The Whitten effect is less pronounced in rats than mice (synchronisation of oestrus in females by exposure to male pheromones), but does occur. Mating usually occurs at night, and a copulatory plug of gelatinous material is left in the vagina for 12–24 h, which then falls out and can be detected to confirm that mating has occurred. Gestation lasts 21–23 days, and can be detected from day 15 by palpation23. The female will start nest building in the later stages of gestation, so appropriate nest building material should be provided. A litter of 6–12 pups is born, often overnight. Pups are very sensitive to rearing conditions, which can influence physiological and psychological development23. In addition, if a female is disturbed during the post-partum period she may destroy her young, so it is very important to minimize disturbances and take extreme care cleaning cages during this time.

Polygamous mating systems are preferred. In these systems one male is housed with two to six females. Pregnant females are removed prior to parturition and returned after weaning. Females in this system produce more milk and have larger litters. Females produce 1–12 litters per year, and if held in a colony females may nurture their young collectively. Alternatively, with monogamous systems, males and females are housed in pairs. The female is mated at the post-partum oestrus, and the young are removed at weaning. This produces the maximum number of litters, but the male may interfere with the young. He can be removed at parturition, and returned to the female after the young are weaned. If the female is lactating during gestation then implantation can be delayed, leading to a 3–7 day increase in the length of gestation. In a variation on this system, a single male is moved between singly housed females, spending a week with each. One male is used for every seven females.

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Growth and development

The pups are born pink and hairless, with eyes and ears closed. They begin to eat solid food from 11 days, and within 14 days the hair coat develops and the eyes open. Weaning occurs from 21 days. Rats grow rapidly in the first 3 months, reaching approximately 50 g at weaning. Adult weight is reached by about 15 weeks, although male rats exhibit prolonged growth, and bones do not become fully ossified until their second year. Male rats can become obese if fed *ad libitum* with insufficient space for exercise, reaching in excess of 500 g. Inbred and outbred rats differ slightly in their rates of growth, outbred strains tending to be larger.

After weaning, young rats should be group housed with plenty of space and enrichment to allow them to play, which has been shown to be essential in the development of normal adult behaviour23.

Handling

In general, rats are amenable animals, which rarely bite if approached correctly, particularly if they are routinely handled using appropriate techniques, although there are strain and sex differences. Approach the animal confidently and restrain it gently but firmly. Animals will typically only bite if stressed or in pain.

1. Remove the lid of the cage, and grasp the rat over the shoulders. Lift the animal from the cage and support it immediately on a non-slip surface, such as the arm or cage top. To avoid injury rats should not be suspended by their tails for any length of time. Once supported on a surface the animal may be sexed by lifting the tail to expose the perineum, as in mice.

2. To restrain the animal, once it has been transferred to a non-slip surface as above, slide the free hand up the animal's body to a position behind the shoulders. Place the thumb under the axilla and between the forelimbs so it rests under the chin to restrain the head, with the fingers behind the other forelimb. The animal may then be lifted, and the hindquarters held with the other hand (see Figure 11.5).

3. Alternatively, for added restraint, complete Step 1 then slide the free hand up the animal's body, but press the elbows with the thumb and fingers to cause the forelegs to cross over under the chin. This method may be used for additional restraint if the animal is aggressive. Restraint should be firm but not too tight as this will impede the animal's respiration.

FIGURE 11.5 Handling the rat. Photo: James and Steve.

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Pain and stress recognition

Rats are naturally curious, and will explore in any new situations. Failure to stand erect and take an interest in their surroundings is an indicator of poor health. They are generally docile but become more aggressive and resist handling during repeated stressful procedures. Acute pain or distress is accompanied by vocalisation and struggling. They will lick or guard a painful area and will sit crouched. Sleep patterns will be disturbed and increased. The Harderian gland, a modified tear gland, produces a red secretion that normally lubricates the eye. When the rat is stressed, this secretion overflows onto the face, producing a red ring around the eye, which is characteristic of stress. This is known as chromodacryorrhoea. Red staining may also appear at the nose, as the secretion flows down the nasolacrimal duct. Rats with abdominal pain may be reluctant to move, and show back-arching, twitching and a cat-like stretch (see www.digires.co.uk/product/pain-assessment-in-the-rat for more details).

Common diseases and health monitoring

Rats are susceptible to a number of diseases which may be zoonotic, have effects on the animals when under stress or affect research data. Some examples are listed in Table 11.3.

Table 11.3 Agents to be included in health-screening protocols for rats[21].

Type of infectious	Examples

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agent	
Zoonoses	Hantaan virus, leptospirosis, *Salmonella* spp.
Disease-causing agents	Sendai virus, pneumonia virus ofmice, rat coronavirus/sialodacryoadenitis virus (RCV/SDAV), *Pasteurella pneumotropica*, *Streptococcus* spp., *Corynebacterium kutscheri*, *Mycoplasma pulmonis*
Diseases affecting research	Kilhamrat virus, rat parvovirus

Biological data and useful reference data

Biological data and useful reference data are shown in Table 11.4.

Table 11.4 Useful data: rat.

Biological data		**Breeding data**	
Adult weight (g)	Male 450–520	Puberty (days)	50–60
	Female 250–300	Age to breed (days)	65–110
Diploid number	42	Gestation (days)	20–23
Food intake	5–10 g/100 g body weight	Litter size	6–12
		Birth weight (g)	5–6
Water intake	10 ml/100 g body weight	Weaning age (days)	21
		Oestrous cycle (days)	4–5
Natural lifespan (years)	3–4	Post-partum oestrus	Fertile
Rectal temperature (°C)	36–40		
Heart rate/min	250–450	**Biochemical data**	
Blood pressure (mmHg)		Serum protein (g/dl)	5.6–7.6
Systole	84–134	Albumin (g/dl)	3.8–4.8
Diastole	60	Globulin (g/dl)	1.8–3
Blood volume (ml/kg)	54–70	Glucose (mg/dl)	50–135
Respiratory rate/min	70–115	Blood urea nitrogen (mg/dl)	15–21
Tidal volume (ml)	0.6–2	Creatinine (mg/dl)	0.2–0.8
		Total bilirubin (mg/dl)	0.2–0.55
Haematological data		Cholesterol (mg/dl)	40–130
Red blood cells ($\times 10^6$/mm^3)	7–10		
PCV (%)	36–48		
Hb (g/dl)	11–18		
White blood cells ($\times 10^3$/mm^3)	6–17		
Neutrophils (%)	9–34		
Lymphocytes (%)	65–85		
Eosinophils (%)	0–6		
Monocytes (%)	0–5		
Basophils (%)	0–1.5		
Platelets ($\times 10^3$/mm^3)	500–1300		

Hamster

Several different species of hamster are used in the laboratory, and in 2010 41[24] hamsters were used in research in the UK8. The commonest hamster used is the golden or Syrian hamster, *Mesocricetus auratus*. These are native to dry regions in Syria and Turkey. Syrian hamsters found in the laboratory are descended from a few animals captured in this region, so captive golden hamsters are highly inbred, and different strains are available. These hamsters are known for their cheek pouches, which they use to gather food for storage in deep burrows, particularly when photoperiod is decreasing. The pouches are unusual in having no lymph drainage, and therefore do not reject tissues transplanted to them. This is useful in immunological and other research. Golden hamsters also have a high incidence of dilated cardiomyopathy, and may be used as models for this condition. Syrian hamsters are often a golden brown colour with a pale underbelly.

The Chinese hamster (*Cricetulus griseus*) originates from Mongolia and north China, where they are found in rocky plains and

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steppes. They used to be more common in the laboratory, but have been largely replaced by the mouse and rat. Some biological products are produced *in vitro* by inserting the relevant gene into Chinese hamster ovary cells. Chinese hamsters are grey-brown, with a black dorsal stripe along the length of the back and a white belly.

Female hamsters are usually larger than males. Syrian hamsters grow to 15–18 cm in length. Chinese hamsters are smaller, reaching 8–12 cm in length, and unlike other hamsters they have a tail 2–3 cm long.

Behaviour

Hamsters are terrestrial animals that live in burrows. In the wild they are crepuscular, but they tend to be nocturnal in the laboratory, sleeping during the day, waking at dusk and then being active through the night. Their sight is poor but their sense of smell and hearing are very acute and they rely heavily on these senses to recognise each other and their environment. Territory marking is done using a secretion from the flank glands, which are visible, particularly in the male Syrian hamster, as dark patches on each side. Hamsters may also be active for short periods during the day.

Housing

Hamsters are essentially solitary animals, particularly Syrian hamsters, which may attack each other. Females will attack males except for a brief period during oestrus, and often attack other females. Groups of same sex animals may be maintained, if they are put together at weaning or before puberty, but aggression may still occur. Chinese hamsters are more sociable, especially males, and will live in groups in the right environment in single sex or mixed sex pairs or groups if put together when young. However, they may still fight once sexual maturity is reached24. Male Chinese hamsters are easier to maintain in groups, but females can be intolerant of each other and may be better living individually.

In the dark period, hamsters are active, fast and agile and therefore require a large cage with plenty of space and enrichment in order to prevent behavioural problems developing. Chinese hamsters in particular are good climbers and will utilise three dimensional space. They can also jump quite high.

Hamsters are adept at chewing through cages, so tough plastic cages with solid bottoms are used, and the small size of Chinese hamsters allows them to escape between the bars of some cages. If they do manage to escape, unlike rats and mice they are unlikely to return to their cages. Deep-piled bedding is usually provided. Little waste is produced.

A breeding female must have deep bedding and soft nesting material, as an inadequate nest will lead to her abandoning or killing her young. To avoid disturbing a nursing mother, cleaning out may be done every 2 weeks.

Feeding

The exact nutritional requirements of hamsters have not been extensively studied. Their stomach consists of a non-glandular part, in which some microbial digestion takes place, and a glandular part. They also have a large caecum which is the main site for microbial digestion. They are coprophagic. In the wild hamsters are omnivorous, eating mostly grains but also shoots, fruit and insects, and hoard food for eating later. Hamsters should be fed a hamster-specific mix if available, although diets formulated for rats and mice may be adequate. These can be supplemented with fruit and vegetables. A diet with 16–20% protein, 5–7% fat and 60–65% carbohydrate appears to be sufficient. Complex carbohydrate (e.g. starch or cellulose) seems to be required and reduces the incidence of non-specific enteritis, known as wet tail17. Some hamsters benefit from the addition of small seeds such as millet, which also provides environmental enrichment. Hamsters eat approximately every 2 h during the active period, consuming 5–7 g of pelleted diet daily.

Syrian hamsters have blunt noses and therefore may have difficulty feeding from some wire-mesh-suspended hoppers, as the mesh is too small. They need hoppers with slots greater than 11 mm, so they can pull the food through onto the floor. Nursing females should be floor-fed, or they become preoccupied with pulling food from the hopper and neglect their young.

Water

Syrian hamsters drink 5–14 ml/100 g of body weight daily (10–20 ml total daily), and Chinese hamsters 11–13 ml/100 g of body weight daily (3–8 ml total)17. Lactating females may need more. Water bottles or automated systems can be used, but the sipper tubes must be stainless steel, as hamsters can bite through glass. For breeding animals, the sipper tubes should extend low into the cage so that neonates can reach them.

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Environment

Hamsters originate from hot, dry countries, and burrow to avoid heat. If unable to burrow, they tolerate heat poorly. Cold may be tolerated better. Hamsters are usually kept between 20 and 24°C with 12–14 h light daily. Hamsters can enter a state of hibernation if the temperature and photoperiod reduce, provided they are alone, have nesting material and have sufficient stored food. If insufficient food is available then hibernation is delayed. Hamsters kept at a constant temperature between 21 and 22°C and minimum 14 h light show reduced seasonal variability, and this may eliminate hibernation[25]. Breeding animals may need to be held at a slightly higher temperature. Relative humidity should be 45–65%11.

Breeding

In Syrian hamsters puberty occurs from as early as 32–42 days, but breeding usually begins at 6–10 weeks for females and 10–14 weeks for males, when they weigh 90–130 g. The female exhibits oestrus every 4 days, and is receptive for a very short time. Fertility is lower in the winter. Gestation in Syrian hamsters lasts only 15.5–16 days, and between five and nine pups are born. Pregnant females should be handled as little as possible. Due to the short gestation period pups are small and immature, weighing just 2–3 g, and an adequate nest is essential to provide warmth and security. Weaning occurs from 21–25 days. Hand rearing of Syrian hamster pups from birth is difficult, so it is not possible to re-derive a colony by Caesarian section to control disease outbreaks. Cross fostering of slightly older pups has been done successfully25.

In Chinese hamsters puberty occurs from 48–100 days, and they are bred from 10–12 weeks. The oestrous cycle lasts 4 days. Gestation lasts 21 days, and 1–11 (average 5) pups weighing 1.5–2 g are born. These are weaned at 21 days, when they weigh 15–17 g.

Female Syrian hamsters are aggressive, and may not tolerate unfamiliar males unless in oestrus. Therefore males and females may be kept separately, and put together for a short period after dark for mating. If the female is not receptive and does not accept the male he should be removed at once. Otherwise he is removed at the end of the dark period, before the light part of the cycle begins. A system of monogamous pairs can be maintained if the male and female are put together at weaning and kept permanently together. Alternatively, females can be rotated through the male's cage at weekly intervals, one male being used for seven females, or a harem can be set up with several males and females together. The females are removed prior to parturition and returned after weaning. However, these systems can lead to fighting. Post-partum mating can occur but it is rare for conception to take place during lactation so this is generally infertile.

Hamsters commonly abandon or kill their young. This can be triggered by environmental disturbances, inadequate nesting material or early handling of mother and young. When disturbed, females may hide their young in their cheek pouches. This affords the young some protection, but it may also suffocate them. To avoid stressing a nursing mother sufficient food and bedding for at least 1 week should be provided just prior to parturition, so that she does not have to be disturbed for 7 days post-partum.

Hamster pups are altricial, being blind and hairless, but in all hamsters the incisors are erupted at birth. In Syrian hamsters the ears open from 4–5 days, the eyes open from 14–16 days and they begin eating solid food from 7–10 days. In Chinese hamsters both eyes and ears open from 10–14 days and they eat solid food from 12 days17.

Handling

Hamsters have a reputation for being vicious, but if handled carefully they are readily tamed and rarely bite unless startled or handled roughly. Males are more docile than females. Chinese hamsters are more easily handled, and they will cling to a finger with all four paws, like a harvest mouse.

Hamsters should be picked up gently but firmly by cupping the animal in the hand. If required, they can be restrained by grasping a *large* pinch of scruff to account for the cheek pouches, and turning over into the hand as for mice. If insufficient scruff is grasped, the hamster will turn and bite. Additional restraint may be achieved by grasping skin along the back between the fingers and palm.

There are no easily accessible veins for dosing and administration in the hamster. Small blood samples can be taken from the saphenous veins, and a technique has been described for cephalic venipuncture for administration of small volumes[26].

Pain and stress recognition

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Hamsters in distress show weight loss, extended sleep period and increased aggression or depression. Ocular discharge and diarrhoea may be associated with stress.

Common diseases and health monitoring

Hamsters suffer from few clinical diseases. They may get non-specific enteritis, or wet tail, which is sometimes associated with *Salmonella* or *Campylobacter* infection, both of which are potentially zoonotic. They can also carry lymphocytic choriomeningitis and Sendai viruses, and these should be included in the regular health-screening programme.

Biological data and useful reference data

Biological data and useful reference data are shown in Table 11.5.

Table 11.5 Useful data: hamster.

Biological data	**Syrian hamster**	**Chinese hamster**
Adult weight male (g)	85–130	30–60
Adult weight female (g)	95–150	25–40
Diploid number	44	22
Food intake (g/100 g body weight)	5–7	3–6
Water intake(ml/100 g body weight)	10–20	3–8
Natural lifespan (years)	1–3	1.5–3
Rectal temperature (°C)	37–38	36–38
Heart rate/min	250–500	325–500
Blood pressure (mmHg)		
Systole	150	
Diastole	100	
Blood volume (ml/kg)	78	
Respiratory rate/min	35–135	74 average
Tidal volume (ml)	0.6–1.4	
Breeding data		
Puberty (days)	32–42	48–100
Age to breed (weeks)	10–12	
Male	10–14	
Female	6–10	
Gestation (days)	15–16	19–21
Litter size	5–9	1–11
Birth weight (g)	2–3	1.5–2
Weaning age (days)	21–25	21
Oestrous cycle (days)	4	4
Post-partum oestrus	Infertile	Fertile

Haematological data		**Biochemical data**	
Red blood cells ($\times 10^6/mm^3$)	6–10	Serum protein (g/dl)	4.5–7.5
PCV (%)	36–55	Albumin (g/dl)	2.6–4.1
Hb (g/dl)	10–16	Globulin (g/dl)	2.7–4.2
White blood cells ($\times 10^3/mm^3$)	3–11	Glucose (mg/dl)	60–150
Neutrophils (%)	10–42	Blood urea nitrogen (mg/dl)	12–25
Lymphocytes (%)	50–95	Creatinine (mg/dl)	0.91–0.99
Eosinophils (%)	0–4.5	Total bilirubin (mg/dl)	0.25–0.6
Monocytes (%)	0–3	Cholesterol (mg/dl)	25–135
Basophils (%)	0–1		
Platelets ($\times 10^3/mm^3$)	200–500		

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Gerbil

There are over 100 species of gerbils recognized. They originate from Africa and Asia, and are are sometimes called desert rats. All species are adapted to dry habitats.

Gerbils have been used in research since the 1950s[27], but are no longer common. Only 560 gerbils were used in the UK in 20108. The species used is usually the Mongolian gerbil, *Meriones unguiculatus*. These are hardy and easy to keep, with few diseases. Mongolian gerbils are typically 110–135 mm long, with a tail approximately 95–120 long. Adult gerbils weigh about 60–130 g, and males are larger than females[28]. The most common coat colours are agouti with a cream belly, black or spotted. Note: the 'spotted' gene is dominant and lethal if homozygous, so spotted gerbils should not be mated with each other to reduce *in utero* mortality of affected pups28.

Gerbils are used in a variety of research areas, including stroke research and studies into seizures. Seizure-prone and seizure-resistant strains of gerbils have been developed.

Gerbils produce a minimum of waste to conserve body fluids, thus they are very clean and have minimal odour.

Behaviour

Most gerbils are diurnal, although some exhibit crepuscular behaviour. They are small and easy to handle, since they are generally docile and rarely bite unless provoked. They are generally very active, and when approached they will resist being caught. Normally they exhibit exploratory behaviour in new surroundings, and if loose they do not hide but show curiosity and interest in the environment. In the wild they are crepuscular. Gerbils are sociable animals, and should be group housed, as breeding pairs with one or more generations of offspring, or in same-sex groups. They use their sense of smell to identify other individuals. Unfamiliar adults caged together will be aggressive. Stable groups may be established by putting animals together preferably at weaning, or by 7–8 weeks. Normal social behaviour will then be seen, in which animals wrestle and groom each other. If introducing unfamiliar gerbils it may be necessary to use a perforated divider in the tank to allow them to become familiar with each other's scent for 5–7 days before mixing them. If aggression is seen, animals should be separated. Aggression may sometimes occur suddenly in previously stable groups.

Gerbils may sit on their haunches to feed or observe their surroundings, and may stand on their toes using their tails for support. They rarely climb.

Housing

Housing designed for hamster or mice may not be suitable for gerbils, since they need access to a burrow system to dig tunnels. If this is not provided, development of normal behaviour can be impaired and they may exhibit abnormal stereotypic behaviours28. They should be provided with a dark nest chamber, accessed via a tunnel, to give the animals an opportunity to burrow. They should also be given material to gnaw, such as wood sticks or straw. Gerbils will also make use of sand baths if provided.

Gerbils can gnaw through plastic hamster and mouse cages, and plastic can cause problems if ingested. They need cages made from suitably strong material to resist attempts to escape. They prefer solid floors to mesh, and need at least 2 cm depth of bedding for nest building, which occurs even if the female is not pregnant. Sawdust or shavings made from pine should not be used, as the fur tends to become matted with these materials. Gerbils need at least 15 cm space between the top of the bedding and the roof of the cage, as they like to sit erect. Gerbils produce very little urine, and their faecal pellets are small and hard, so they are easy to keep clean.

Feeding

Gerbils have a simple stomach, and the caecum and colon are not especially well developed. Their natural diet consists of low-fibre foods such as seeds17. They are omnivorous, hoarding rodents, able to survive on a variety of foodstuffs including fresh and dried plant matter, tubers, bulbs, roots, seeds and insects depending on availability. In the wild they gather seeds and other foodstuffs from August onwards and store this in their burrows to last them through the winter. This enables them to thrive in dry habitats, where vegetation is sparse. They will gather food for as long as the weather is good, often continuing until winter. During winter gerbils may stay underground existing on their food stores.

Gerbils are coprophagic. Eating is spread throughout the day and night. Standard rodent diets with 22% protein are adequate, but a dietary fat level below 4% is recommended, to prevent obesity and high blood cholesterol. Obesity can lead to infertility in

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females due to fat deposition around the genital tract. Standard food hoppers are normally used, but supplementary food may be put onto the floor for young gerbils until they become used to the hoppers. Pellets can also be soaked. Gerbils consume 5–8 g/100 g of body weight pelleted food daily (5–6 g)17,28.

Water

Gerbils, being desert animals, can survive with very little drinking water, although older males and reproducing females need more than younger animals. They produce very concentrated urine, and are resistant to water loss. Nonetheless, plentiful drinking water should be provided in bottles or automated systems. Gerbils consume 4–7 ml/100 g of body weight water daily28.

Environment

Gerbils can tolerate a wide range of environmental conditions. At high temperatures they can regulate their body temperature by behavioural means if given the opportunity to burrow. They should be kept between 20 and 24°C, with humidity between 35 and 55%11: at higher humidities they develop matted fur. A period of 12 h light should be provided daily at 350–400 lux.

Breeding

In the wild, gerbils live in groups consisting of a breeding pair and several generations of offspring, and breeding is suppressed in the mature offspring. It is thought that by staying with their parents young gerbils develop parenting skills, improving their own reproductive performance subsequently28. Gerbils breed all year round in the laboratory, but are less-efficient breeders than other rodents. If a long-time partner dies, the remaining partner may not breed again. Puberty occurs from 6 weeks of age, and they are usually bred from 9–12 weeks. Successful breeding can be achieved by pairing a male and female between 60 and 90 days of age, and never separating them: if they are separated then reintroduced, they may fight. An alternative system is a harem system, with two or more females grouped with a single male. Oestrus occurs in the female every 4–6 days. Gestation lasts 24–26 days, unless the female is mated at the post-partum oestrus. In this case, lactation prolongs the gestation period to 27–48 days, resulting in an interbirth interval of 29–35 days. To avoid post-partum mating the male can be removed at parturition, but the separation should be for less than 2 weeks. Three to seven pups are born, weighing 2.5 g, and the male assists in nest building and caring for the pups. Neonatal mortality is high, up to 20%28. Pups are born blind, deaf and hairless. Hair appears from 5 to 7 days, ears open at 12–14 days, teeth erupt from 10 to 16 days and eyes open from 16 to 20 days. Pups begin to eat solid food from 16 days, and weaning takes place at a minimum of 21 days, when pups weigh 14–18 g.

Handling

Gerbils may be restrained using techniques as for mouse and rat. They readily run into a tube, or may be picked up in cupped hands. Great care must be taken if lifting by the tail: the body must be supported immediately, and if the tip of the tail is held the skin may slip off.

Pain and stress recognition

Healthy gerbils are inquisitive and active. If unwell, gerbils may show weight loss, piloerection with a characteristic scruffy appearance, and a hunched posture. Ocular discharges and diarrhoea may be associated with stress. Changes in behaviour, such as increased aggression or depression, increased respiratory rate, diarrhoea or constipation may also be seen.

Common diseases and health monitoring

Gerbils suffer from relatively few clinical diseases. They can develop Tyzzers disease, a frequently fatal enteric infection which also affects the liver, and can also carry lymphocytic choriomeningitis and Sendai viruses. Between 20 and 50% of gerbils may exhibit seizures. These may be triggered by fear, handling or a new environment. Seizures may be mild or severe, and animals may occasionally die. This trait can be used for studies of epilepsy. Should this occur, the best treatment is to leave the gerbil in a warm, dark, quiet place to recover. Frequent handling from an early age will reduce the frequency of seizures.

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Biological data and useful reference data

See Table 11.6.

Table 11.6 Useful data: gerbil.

Biological data		**Breeding data**	
Adult weight (g)	Male 80–130 Female 60–100	Puberty	6 weeks
		Vaginal opening	41 days (28 g)
Diploid number	44	Age to breed male (days)	70–85
Food intake	5–8 g/100 g body weight	Age to breed female	65–85 (days)
Water intake	4–7 ml/100 g body weight	Gestation (days)	24–26 (27–48 if lactating)
Natural lifespan (years)	3–4	Litter size	3–7 (average 4.5)
Rectal temperature (°C)	37–38.5	Birth weight (g)	2.5–3.5 (depends on litter size)
Heart rate/min	360		
Blood volume (ml/kg)	66–78	Weaning age (days)	21–24
Respiratory rate/min	90	Oestrous cycle (days)	4–5
		Post-partum oestrus	Fertile
Haematological data			
Red blood cells ($\times 10^6$/mm^3)	8–9*	**Biochemical data**	
		Serum protein (g/dl)	4.3–12.5
PCV (%)	43–49	Albumin (g/dl)	1.8–5.5
Hb (g/dl)	12.6–16.2	Globulin (g/dl)	1.2–6
White blood cells ($\times 10^3$/mm^3)	7–15	Glucose (mg/dl)	50–135
		Blood urea nitrogen (mg/dl)	17–27
Neutrophils (%)	5–34		
Lymphocytes (%)	60–95	Creatinine (mg/dl)	0.6–1.4
Eosinophils (%)	0–4	Total bilirubin (mg/dl)	0.2–0.6
Monocytes (%)	0–3	Cholesterol (mg/dl)	90–150
Basophils (%)	0–1		
Platelets ($\times 10^3$/mm^3)	400–600		

*High levels of reticulocytes, stippled red blood cells and polychromatic cells.

Guinea Pig

The guinea pig, *Cavia porcellus*, originates from South America, where they are still sometimes reared for their meat. The domestic guinea pig as such is not found in the wild, and is probably a descendent of a related species of cavy[22,29]. Guinea pigs have been extensively used as research subjects, but have now been largely replaced by rats and mice. In 2010, 13 586 guinea pigs were used in research in the UK8, mainly for applied studies, such as allergic responses, scurvy and tuberculosis. There are several different varieties of guinea pig available, including the short hair (English and American varieties), Abyssinian (which have hair in whorls) and Peruvian (which have long hair). Commonly used laboratory stocks are derived from the short-hair variety. The albino Dunkin–Hartley outbred stock is the most common type of guinea pig used. Strains 2 and 13 are inbred strains.

Behaviour

Guinea pigs are amenable animals which show little aggression and rarely bite. Naturally guinea pigs live in breeding groups consisting of one adult male and several females with their offspring. In the wild, male offspring leave the colony when mature, but in the laboratory they may become integrated into the group with little aggression[30]. They develop a stable dominance hierarchy, which is maintained mainly by olfactory cues, but with some barbering and chewing of subordinate males. Males mark females with secretion from their anal glands, and both sexes mark the environment with this secretion. Urine is also important in communication.

In small populations, linear dominance hierarchies develop in both sexes, the dominant male fathering most of the offspring.

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As the population increases above 10–15 individuals these split into subgroups consisting of one to four males and one to seven females, dominance hierarchies developing within each subgroup30. Each subgroup occupies a territory, with minimal overlap between the territories of adjacent subgroups. These groups are stable over time, and social stress and aggression are minimal in these circumstances, allowing guinea pigs to live in groups even at high stocking densities. Even unfamiliar animals can be grouped with minimal aggression, as a dominance hierarchy usually establishes quickly. Occasionally, fighting may ensue between unfamiliar adult males, particularly in cramped conditions or if oestrous females are present. If this is the case then the subordinate males should be removed.

Guinea pigs in the laboratory are active for periods throughout the day and night, with rest periods in between30. When startled, they either become immobile, or stampede and vocalize. This may lead to trampling of young and makes capture difficult. Providing bolt-holes and barriers within the pen and frequent handling to habituate the animals reduces this behaviour. The approach of a handler causes excitement, and guinea pigs should exhibit a scatter reaction as an attempt is made to pick them up. It is normal behaviour for a guinea pig to 'resist arrest' and vocalise strongly. If this does not occur it may indicate that there is a problem.

Guinea pigs are creatures of habit and become increasingly unable to cope with changes in routine as maturity approaches. If there are any changes in the type of food hopper or water bottle, or in the type of food or water, the guinea pig may be unable to adapt and cease eating and drinking. This is particularly disastrous with pregnant females. Similarly, if there are changes in the type of housing, problems may be encountered.

Housing

Guinea pigs are gregarious and should be housed in groups, consisting of a harem (one or two males with breeding females), pairs, or mixed-sex or all female groups. Solitary housing or single-sex groups of more than two males should be avoided.

They may be kept in floor pens, or large plastic or steel cages. Although guinea pigs rarely jump, cages should have sides at least 23 cm high to prevent escapes, and more height is required for open-topped floor pens (Figure 11.6). Guinea pigs thrive on solid floors with substrate. Mesh floors predispose to foot-pad ulcers and increased stress levels, and are not recommended.

FIGURE 11.6 Guinea pigs housed in floor pens.



Bedding materials provide comfort and a substrate for rooting behaviour. Materials such as wood shavings, paper-based bedding, ground corn cobs or sawdust may be used, together with hay. Fine shavings and sawdust alone may cling to moist areas, such as the perineum, and probably are best not given to breeding guinea pigs. Larger shavings are better for these animals. Guinea pigs are messy animals and will disperse opaque, creamy coloured urine and faecal pellets throughout the pen, requiring frequent

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cleaning.

Feeding and water

Guinea pigs are herbivores, their natural diet consisting mainly of grass. They are unique among non-primates in having a dietary requirement for vitamin C, and need a diet specifically formulated for guinea pigs, not one designed for any other species. They need approximately 10 mg vitamin C daily, and 20 mg if pregnant. This can be supplied in the food or water, or by giving cabbage, kale or oranges. Vitamin C in pelleted diet degrades within 90 days, so food must be stored correctly and used within the recommended shelf life to avoid deficiency. Supplements of hay may be given, and this also provides environmental enrichment. The food requirement is 6 g/100 g body weight daily. However, because much of the food is wasted more should be supplied. The food should contain 18–20% crude protein and 10–16% fibre. Coprophagy does occur in the guinea pig, but may not be essential. Guinea pigs are fastidious eaters and will reject unfamiliar food[31].

Food and water bowls placed on the floor soon become soiled, so suspended hoppers are recommended. Guinea pigs also tend to play with drinkers, leading to wet bedding, and bottles quickly become empty. Automated watering systems ensure a constant water supply, but in solid-floored systems, care must be taken to prevent flooding. Any changes in watering system will upset the routine, and the guinea pig will need help to adapt. The water requirement is 10–14 ml/100 g body weight daily.

Environment

Guinea pigs thrive at temperatures between 18 and 24°C, with a humidity of 40 and 70%. They are better able to withstand cold than heat31.

Breeding

Guinea pigs can breed all year round, with peaks in spring. Female guinea pigs can reach puberty as early as 30 days, and males from 2–3 months. Pairing should take place when the female is 400 g (at 2–3 months), and the male 650 g (3–4 months). One boar can be housed with one to ten females. The oestrous cycle of the female lasts 15–17 days, and she is receptive for 6–11 h. The vagina is covered by an epithelial membrane, except during oestrus and parturition, both of which are signalled by perforation of the membrane. Gestation lasts 59–72 days (average 63–68 days), depending on litter size. In the last week, the pubic symphysis separates under the influence of the hormone relaxin, and once the gap reaches 15 mm parturition will take place within 48 h. Females should have their first litter before reaching 7–8 months of age, or the symphysis will be unable to separate sufficiently and dystocia (difficult birth) will result. In any case, there is a high incidence of abortion, dystocia and stillbirth. In smaller litters, pups are larger and dystocia is more common. Female guinea pigs can breed until they are 20 months old. Thereafter, the litter size tends to drop and dystocia is more common.

Growth

Between one and six pups are born. Neonatal guinea pigs are precocious, weighing 60–100 g, with hair, teeth, claws and partial eyesight. They are mobile at birth, and begin eating solid food within a few days, while continuing to suckle. Growth depends largely on the strain of guinea pig. Young guinea pigs should typically gain 2.5–3.5 g daily up to 60 days. Weaning takes place at 180 g (15–28 days), or 21 days (165–240 g). Weaned males intended for breeding need to be weaned late or group housed to allow development of normal adult reproductive behaviour.

Hand rearing is not difficult, making Caesarian re-derivation of colonies quite easy. Also the young may be hand reared to allow the sow to be mated at the post-partum oestrus. The young are not hungry until 12–24 h after birth, and can then be fed cows' milk or soaked guinea pig pellets.

Handling

Guinea pigs are easily startled, and they vocalise and try to avoid capture when approached. The handler's movements should be rapid and smooth, to avoid frightening the animal. The animal should be grasped quickly and smoothly, placing the thumb and fingers of one hand on either side of the shoulders, then lifted and the free hand placed beneath the hindquarters to support the weight. The thumb can be placed under the jaw for additional restraint. The guinea pig can then be turned over for intraperitoneal

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injections or sexing. Holding the animal against the handler's body or positioning the thumb under the foreleg and beneath the chin as in the rat can provide additional restraint. Alternatively, one hand may be placed under the thorax and the other under the rear feet. It is particularly important to support pregnant females with two hands (see Figure 11.7).

FIGURE 11.7 Handling the guinea pig.



Recognition of pain and stress

Guinea pigs are alert, apprehensive animals that will try to avoid capture and restraint, and loud vocalization accompanies even minor procedures. They are stoical animals, and they mask signs of pain or distress until the disease process is advanced. It can be difficult to assess whether they are in distress. Any unusual sign of acceptance indicates the animal is unwell. They often appear sleepy when in pain and rarely show aggression.

Common diseases and health monitoring

Relatively few infectious diseases are seen in guinea pigs. Non-infectious diseases such as scurvy or diarrhoea may occasionally be seen if the diet is inadequate. Most infectious diseases seen in guinea pigs are bacterial, with abscesses and non-specific infections most commonly encountered. They may also develop respiratory infections. Guinea pigs can carry lymphocytic choriomeningitis virus (a zoonosis) and Sendai virus, and these two antigens should be included in regular screening programmes.

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External parasites (lice, mites, fungi) are rare.

Anaesthesia

These are probably the most difficult rodents in which to achieve safe and effective general anaesthesia. The response to injectable agents is variable and post-anaesthetic complications, such as respiratory infection, generalised depression, inappetance and digestive disturbances, may frequently be seen. Many of these problems may be avoided by careful selection of anaesthetic agents and high standards of pre-, intra- and post-anaesthetic care.

Biological data and useful reference data

See Table 11.7.

Table 11.7 Useful data: guinea pig.

Biological data		**Breeding data**	
Adult weight (g)	Male 850–1200 Female 700–900	Puberty (days)	Male 60 Female 30
Diploid number	64	Age to breed male	3–4 months, 600–700 g
Food intake	6 g/100 g body weight	Age to breed female	2–3 months, 300–450 g
Water intake	10–14 ml/100 g body weight	Gestation (days)	59–72
Natural lifespan (years)	4–8	Litter size	1–6
Rectal temperature (°C)	37.2–40	Birth weight (g)	70–100
Heart rate/min	230–380	Weaning age (weeks)	3–4
Blood pressure (mmHg)		Oestrous cycle (days)	15–17
Systole	80–94	Post-partum oestrus	Fertile
Diastole	55–58		
Blood volume (ml/kg)	69–75	**Biochemical data**	
Respiratory rate/min	42–104	Serum protein (g/dl)	4.6–6.2
Tidal volume (ml)	2.3–5.3	Albumin (g/dl)	2.1–3.9
		Globulin (g/dl)	1.7–2.6
Haematological data		Glucose (mg/dl)	60–125
Red blood cells ($\times 10^6$/mm^3)	4.5–7	Blood urea nitrogen (mg/dl)	9–31.5
PCV (%)	37–48	Creatinine (mg/dl)	0.6–2.2
Hb (g/dl)	11–15	Total bilirubin (mg/dl)	0.3–0.9
White blood cells ($\times 10^3$/mm^3)	7–18	Cholesterol (mg/dl)	20–43
Neutrophils (%)	28–44		
Lymphocytes (%)	39–72		
Eosinophils (%)	1–5		
Monocytes (%)	3–12		
Basophils (%)	0–3		
Platelets ($\times 10^3$/mm^3)	250–850		

Rabbit

The laboratory rabbit belongs to the order Lagomorpha (meaning 'hairy ears'), and is derived from the domestic rabbit, *Oryctolagus cuniculus*. Many breeds of rabbit are available, ranging from the large giant chinchilla and Flemish giant, which may reach over 7 kg in weight, to the Dutch and Polish breeds which may reach no more than 0.9 kg. The commonest breed seen in the laboratory is the large outbred New Zealand white. Some inbred strains are available. Lagomorphs are characterized by having six incisors, whereas rodents have only four. An additional small pair is present behind the main upper incisors.

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Rabbits are prey animals and have acute senses. Their large ears are highly vascular and are used for heat regulation and sound gathering. They have sensory hairs on the upper lips (vibrissae), a good sense of smell and their eyes are prominent and occupy a lateral position on the head, allowing them 360° vision. They have good vision in low light levels.

Their teeth are open rooted, and grow 10–12 cm each year. The skeleton of the rabbit is fragile, comprising only 8% of body weight. Their long bones and lumbar spine are particularly prone to fracture, especially if handling is poor[32].

Behaviour

Wild rabbits are crepuscular or nocturnal, with clear daily patterns of activity. They emerge from their burrows at dusk, graze through the night and return to their burrows early in the morning. Their physiological parameters also show daily patterns: locomotor activity, food and water intake, expulsion of hard faeces and urine excretion all occur mainly in the dark phase. However, rabbits are very sensitive to external influences and their circadian rhythms may be entrained by light or feeding. Therefore, unless disturbances from husbandry or procedures are minimal, they often become diurnal, and periods of activity may be seen throughout the day and night[33].

Wild rabbits live in social groups, the size of which changes depending on the season. In the winter they live in small, mixed-sex social groups of two to ten animals, defending small territories[34]. They may disperse during the summer months. Both males and females develop dominance hierarchies, although it is less obvious in females and may only be seen during competitive situations. In small groups, monogamous pairs are most common, whereas in larger burrows with multiple animals, promiscuity is often seen[35].

Adolescent rabbits may leave the natal group at the end of breeding season and venture into different warrens[36]. Females may disperse for a chance of gaining a higher rank in a new territory, and males may disperse to find a mate. They are not forced out of their natal units due to aggression, but simply choose to leave.

Rabbits are very territorial, and they mark their territory through faeces, urine and glandular secretions from under their chins onto objects within their environment. They choose territories with plentiful food resources and ample cover, to provide protection from potential predators and high temperatures[37].

Rabbits communicate mainly by smell. They mark their territory with secretions from their submandibular (chin) gland and anal glands, urine and dung hills[38]. These consist of large faecal pellets that are covered with secretion from the anal glands[39]. Males, both dominant and subordinate, visit the latrine sites more frequently than females, since their main duty is to guard the territory39. ‘Chinning’ involves rubbing their chin against an inanimate object while giving off scent from the chin gland. This is done for territory marking and to indicate whether they are willing to mate[40]. During breeding there is increased defence of the territory.

Housing

Rabbits are social animals, which are able to utilise a complex, three-dimensional environment. If given sufficient space, laboratory rabbits will exhibit the full range of behaviours seen in their wild ancestors, including standing on their hind legs, climbing up to a good vantage point, exploratory and tunnelling behaviour, and social activity. It is essential to provide sufficient space for the animals to perform these behaviours, to prevent boredom and skeletal problems. Cages should allow the rabbit to stretch out in at least one direction and be tall enough for the animal to stand, and include a box or shelf they can sit on. Environmental enrichment can be provided by giving hay, chew sticks or cardboard boxes to play with, and animals can be taken to exercise areas for short periods.

Overeating, leading to obesity, and overgrooming, leading to the formation of gastric hairballs, may be stereotypic behaviours, and are often associated with boredom. If there is insufficient opportunity for exercise, there will be prolonged periods of inactivity and increased stereotypic behaviour. There may be hypoplasia of bone tissue and osteoporosis, and an increased risk of fractures and nerve damage leading to cage paralysis. These problems are seldom seen in floor-housed rabbits. Single caging should be used only where group housing is inappropriate, such as for adult males, or if an animal needs to be isolated for measurement of food and water intake. Pair housing in large cages may be an acceptable alternative in many circumstances.

Aggressive behaviour may be seen if incompatible animals are housed together. They may chase each other, tail flag, urinate, squeal and fight. The incidence of aggressive behaviour depends on many factors, including strain (Dutch rabbits are more aggressive than New Zealand Whites), sex, age and weight, pen size and construction, the relatedness of the individuals and the proximity of other rabbits of the opposite sex. Aggressive behaviour is seen most in breeding and pubertal animals, and adult males. Males are most aggressive when competing for food, territory or females. It is common for them to be separated from 10

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weeks of age to prevent fighting.

Rabbits should always be housed where they can see other rabbits. Solid floors with bedding are preferred: grid floors predispose to sore hocks (pododermatitis), and high ammonia levels can trigger the development of respiratory disease.

Groups of compatible rabbits, such as breeding females, a doe and litter, single-sex groups of newly weaned animals and stable groups of animals on procedure, can be housed in floor pens. Animals to be group housed should be of similar weights, and it is important to provide ample dividers, cardboard boxes or large tubes within the pen so nervous or frightened animals have bolt-holes where they can hide from aggressive conspecifics. Continued monitoring of newly formed groups for excessive aggression is required.

Group housing in pens has many advantages over single cages (see Figure 11.8). Group-housed animals generally have improved physical and psychological well-being. Animals can exhibit natural behaviour and interact socially, which reduces stereotypy; they tend to be calmer and more docile, the increased opportunity for exercise reduces osteoporosis and cage paralysis, and improved ventilation leads to fewer respiratory problems. However, there may be increased aggressive behaviour and stress in unstable and incompatible groups, identification and treatment of individuals is more difficult and exposure of staff to soiled bedding may increase the risk of allergy. Females group housed after puberty may develop pseudopregnancy, which can become a problem in breeding colonies. This will usually resolve once the animal leaves the group.

FIGURE 11.8 Group-housed rabbits in floor pens.



Rabbits feel secure and confident if surrounded by their own smell, and are disturbed by excessive cleaning or the use of strong smelling disinfectants. Over-frequent cleaning simply results in increased territory marking and upsets the animals' confidence. Floor pens should be deeply lined with bedding materials, which do not have a strong smell. The rabbits will urinate and defaecate in latrine areas, but faecal matter will become spread throughout the pen.

Rabbits produce copious, turbid urine, which may be yellow to dark red, due to the presence of a varying quantity of porphyrins.

Feeding

Rabbits are coprophagic. The digestive system includes a large glandular stomach, a long small intestine and an enlarged caecum, which contains complex microbial flora and is the main site of cellulose digestion. Food remains in the stomach for several hours before being gradually introduced into the small intestine. Undigested particles pass into the caecum, where microbes continue digestion. Caecal contents gradually enter the colon, where they become surrounded by mucus and form into a string of soft pellets known as caecotrophes. These are excreted during the dark phase and are swallowed directly from the anus. The now

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digested pellets pass through the small intestine again, where nutrients are absorbed. Any waste matter remaining is then expelled as hard faecal pellets that are seen in the environment. It is rare to see caecotrophs: these may mean that the diet is unsuitable or that the rabbit is over-eating. The digestive process can last around 24 h from beginning to end. Rabbits naturally feed mainly on roughage, and this should form the main component of the diet. If the fibre level is inadequate, this may lead to gut problems such as mucoid enteritis. Fibre should comprise as much as 22.5% especially for long-term maintenance: this allows feeding *ad libitum* without risk of obesity, and also reduces the risk of hairballs or diarrhoea. Lower fibre levels may be acceptable for breeding and growth. A diet consisting mainly of good-quality roughage (straw or hay) is adequate. If a rabbit is fed on pellets or mixed cereals, good-quality hay should supplement the daily ration. Otherwise, unless the amount is restricted the animal can overeat and become overweight[41].

Roughage keeps the gut working efficiently and wears down the constantly-growing teeth. *Ad libitum* nibbling of hay prevents boredom and behavioural problems. Small quantities of concentrates (cereals, seeds, etc.) and fresh vegetable matter may also be given for variety. Grass may be suitable for this purpose: it has very high fibre level (around 20%) and around 14% protein.

A diet with 12–22% fibre, and 12% protein for maintenance or 15–17% protein for growth is recommended. Rabbits need 5 g of high-energy food per 100 g body weight daily (100–300 g diet daily).

As gut flora play an important part in digestion, changes in diet should be done gradually, over a 4–5-day period, to allow the flora to adapt. Failure to do this will result in diarrhoea or anorexia. Newly acquired animals should be fed on the diet that they are used to and change should be gradual. Rabbits can be very fussy and may even starve rather than accept a different product.

Water

Rabbits consume about 10 ml water per 100 g body weight daily (except in lactating does, which will consume up to 90 ml/100 g body weight, sometimes more than 1 litre per day). Water should be supplied *ad libitum*. Rabbits have a tendency to play with water bottles, so they should be checked frequently to ensure they are not empty, and that the floor has not become wet.

Environment

Rabbits require temperatures between 15 and 21°C. Neonates cannot maintain their body temperatures until they are 7 days old, so they must be kept in a warm environment. Humidity should be kept between 45 and 65%. Females require 14–16 h of light daily, and males 8–10 h, with or without periods of twilight. Shorter light cycles may result in reduced sexual activity in the autumn. Low-intensity light should be provided for albino animals. Rabbits are sensitive to high-pitched sounds, hearing from 75 to 50 000 Hz[42], so care should be taken not to expose them to excessive ultrasound. Background noise can help to prevent the animals from being startled by sudden noises. Ventilation is particularly important for rabbits. Poor ventilation, allowing a build-up of ammonia, or low relative humidity levels predispose to respiratory diseases.

Breeding

Puberty occurs from 3–4 months in dwarf breeds, later in large breeds. Females reach puberty earlier than males: females begin breeding at 4–9 months, males at 6–10 months. Rabbits are induced ovulators, and have no oestrous cycle as such. They are receptive for 7–10 days, then inactive for 1–2 days. When receptive, the vulva may be red and swollen and the doe stands to be mounted. Does are also receptive at intervals during pregnancy and lactation. There is some seasonal effect, and ovarian activity diminishes as days shorten[43]. If day length is maintained at 16 h/8 h or 14 h/10 h light/dark, with a high calorie intake and elevated environmental temperature, the seasonal effect will be suppressed. Does may breed until they are 3 years old or more. Breeding problems reduce if doe is less than 6 months old when first bred. A doe can produce eight litters/year, but breeding efficiency falls after 7–11 litters, or 3–4 years.

Various mating systems exist. Group-housed does can be taken individually to the buck, or a buck may be taken to a group of two to five does, and removed after mating or after a few minutes if mating does not occur. Coitus induces ovulation approximately 10 h post-mating, and results in pregnancy lasting 31–32 days in 75% of does. Care should be taken in handling the doe during gestation, as the pregnancy is easily aborted.

The doe must have a clean nest box in the last week of gestation. The doe will pull fur from her flank to line her nest. Does usually kitten in the morning, and between four and ten kits are born, although this varies with the breed. Parturition is very rapid, taking 10 min or less. Dystocia is rare but some young may be stillborn or rejected from the nest. Litters retained more than 34 days are usually stillborn. Rarely, the kits may be eaten by the doe if there is a deformity or they are dead, but it may also happen

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if the doe is inexperienced or if there is some environmental disturbance. A doe will scatter her young if the nest is dirty, or if it smells of disinfectant. Does may be rebred shortly after parturition, but more usually are mated after weaning.

Growth

Newborn rabbit kits are altricial, naked with closed eyes and ears, and receive very little care from their parents. They are only suckled for 5–10 min once daily, in the morning. This is sufficient because rabbit milk has a high nutritive value. Kits can miss a feed and survive 48 h, and older kits can survive two missed feeds. Kits will drink from any lactating doe and at any time of day or night so fostering is relatively easy.

The doe does not brood the kits, clean them or retrieve them if they stray from the nest. The kits remain quiet in the nest, covered with nest material between feeds. Then, 1–2 h before feeding, their activity gradually increases, they become exposed, and they become very sensitive to disturbances. When the doe arrives, the kits lift their heads up and search for nipples. This allows for very rapid feeding: they can consume 25% of their body weight in a few minutes.

Kits respond to auditory stimuli from day 7. The eyes open at day 9–10, and they begin to leave the nest from 13–18 days. They start to eat dry food from 3 weeks, and should be weaned at 6–8 weeks. Hand rearing of rabbits is relatively easy.

Handling

Rabbits should always be approached in a calm and confident manner. They are generally placid and amenable, and can be readily trained. They rarely bite, but can inflict painful wounds with their sharp claws. They can become accustomed to frequent handling, which facilitates the performance of procedures. There are a number of particular considerations when handling rabbits. The animal should *never* be lifted by the ears, and the back must be supported at all times, otherwise contractions of the strong spinal muscles during struggling can result in injuries to the spine. Rabbits have powerful hind legs, and the handler must be careful to avoid being kicked or scratched. Before removing a rabbit from a cage or floor pen, orientate the rabbit so it is facing you. Grasp the animal by the scruff, avoiding the ears, and lift the front end. Then either slide the free hand beneath the rabbit, placing the thumb and little finger in front of the hind legs to extend them and lift the animal, or place the free hand behind the animal's hindquarters and scoop the animal towards you. Once the animal has been lifted clear of the cage or pen, it should be carried held head uppermost against the chest, or resting on the arm with its head tucked under the armpit, with the scruff securely held and the back and hindquarters supported (see Figure 11.9).

FIGURE 11.9 Handling the rabbit. The rabbit is held with its head tucked under the handler's arm and with the back and hindquarters supported by the handler's forearms.

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Pain and stress recognition

Rabbits often react to painful procedures with stoic acceptance. This may relate to feral behaviour where concealment is important for survival. A rabbit in pain may be isolated from the group. There may be reduced food and water intake and limited movement. Abdominal pain may cause twitching and writhing, but this is often very subtle. There may be apparent photosensitivity and ocular discharge with protrusion of the third eyelid. Faecal staining of the coat, digestive disturbances and dehydration may also be seen.

Common diseases and health monitoring

The most significant problems seen in rabbits are husbandry-related. It is very important that the diet, housing, environment and general management are of a high standard. Obesity and hairballs are associated with boredom, cage paralysis can occur if there is inadequate exercise, and digestive disturbances can result from lack of fibre or sudden changes in diet.

Rabbits suffer from very few viral diseases, but myxomatosis and viral haemorrhagic disease of rabbits are both potentially fatal. Myxomatosis is common in wild and pet rabbits, and can be brought unwittingly into the laboratory by personnel who have contacted the virus outside or by insect vectors. Viral haemorrhagic disease of rabbits is often characterised by sudden death. It is currently rare in laboratory-reared rabbits.

Rabbits suffer from a number of bacterial infections, the most important of which is *Pasteurella multocida*. This is mainly a respiratory pathogen, which can be carried subclinically in the upper respiratory tract, causing problems when the animal is stressed. Treatment may reduce the symptoms of *Pasteurella*, but it will not eradicate it. If this organism is present, it is essential to maintain adequate ventilation, particularly humidity, ensure meticulous hygiene, keep the stocking density low and avoid stress.

Many rabbit populations are infected with *Encephalitozoon cuniculi*, a protozoan parasite. This is often subclinical, or may be associated with a variety of clinical signs including ill- thrift and neurological signs. It is transmitted in urine.

Diarrhoea is also common. Causes include sudden diet changes, infestation with intestinal parasites such as coccidia and other infectious causes. Mucoid enteropathy occurs in growing rabbits. It seems to be associated with an inadequate level of dietary fibre and stress. Inappetance is another commonly encountered problem, associated with environment or diet changes, hairballs or subclinical diseases. This can cause a rapid loss of condition and should be treated promptly.

Acquisition of rabbits from disease-free stock, an appropriate barrier including quarantine periods and appropriate protective clothing, and a good standard of hygiene are essential to prevent these diseases.

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Biological data and useful reference data

See Table 11.8.

Table 11.8 Useful data: rabbit[44].

Biological data		**Breeding data**	
Adult weight (g)	900–6000	Puberty (days)	90–120
Diploid number	44	Age to breed male (months)	6–10
Food intake	5 g/100 g body weight	Age to breed female (months)	4–9
Water intake	10 ml/100 g body weight	Gestation (days)	30–32
Natural lifespan (years)	6–12	Litter size	4–10
Rectal temperature (°C)	38.5–40	Birth weight (g)	30–70
Heart rate/min	130–325	Weaning age (weeks)	4–8
Blood pressure (mmHg)		Oestrous cycle	Induced ovulator
Systole	90–130	Post-partum oestrus	Fertile (not used)
Diastole	60–90		
Blood volume (ml/kg)	57–65	**Biochemical data**	
Respiratory rate/min	30–60	Serum protein (g/dl)	5.4–7.5
Tidal volume (ml/kg)	4–6	Albumin (g/dl)	2.7–4.6
Haematological data		Globulin (g/dl)	1.5–2.8
Red blood cells ($\times 10^6$/mm^3)	4–7	Glucose (mmol/l)	5.5–8.2
PCV (%)	36–48	Blood urea nitrogen (mmol/l)	4.6–10.7
Hb (g/dl)	10–15.5	Creatinine (umol/l)	74–171
White blood cells ($\times 10^3$/mm^3)	9–11	Total bilirubin (mg/dl)	0.25–0.74
Neutrophils (%)	20–75*	Cholesterol (mg/dl)	35–53
Lymphocytes (%)	30–85		
Eosinophils (%)	0–4		
Monocytes (%)	1–4		
Basophils (%)	2–7		
Platelets ($\times 10^3$/mm^3)	250–650		

*Neutrophils often resemble eosinophils due to cytoplasmic granules.

Anaesthesia in the rabbit

Rabbits have a small lung volume, and are susceptible to respiratory depression. Stress prior to induction of general anaesthesia can lead to cardiac and respiratory arrest. It is therefore very important to keep stress to the absolute minimum, before and after anaesthesia. Rabbits may develop gut stasis after anaesthesia and may need treatment with probiotics or drugs to encourage the restoration of gastrointestinal motility.

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Chapter 12

Carnivores

The overwhelming majority of animals used in research are rodents or fish. However, some studies require the use of higher species, including carnivores. In 2010, 5319 carnivores were used in research in the UK, mainly for applied studies such as safety testing of human drugs and development of veterinary medicines. This represents less than 0.15% of the total number of animals used in research[1]. Approximately 3727 of these animals were dogs, with cats and ferrets accounting for much of the remainder (152 and 727 respectively). Since the use of cats is negligible this species will not be considered further in this book. Further information can be found in *The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals*, 8th edn (edited by R Hubrecht and J Kirkwood, Wiley-Blackwell, 2010).

Dogs and cats are familiar as companion animals, and are afforded particular protection under the Animals (Scientific Procedures) Act 1986. The most appropriate animal for the studies to be undertaken will be selected according to criteria including species most likely to produce accurate and relevant results, biological and behavioural characteristics, least number of animals required and practical and economic considerations[2]. If companion animals are selected for use, particular and additional scientific justification will be required before their use will be authorised. In general, they may only be obtained from licensed breeding establishments, although in particular circumstances animals from the general population may be used. For example, clinical cases may be used with the permission of the owners for research into the efficacy of treatments or control of pain after surgery.

Dog

The domestic dog, *Canis familiaris*, has been domesticated for thousands of years. Other members of the genus include jackals and wolves, and dogs are thought to have descended from a Eurasian subspecies of the grey wolf[3]. Dogs have been used in research for many centuries; for example, in the 1600s, William Harvey used them in his studies of the circulation[4]. They have been popular for research in the past as they are cooperative, they are a suitable size for testing of paediatric devices and protocols for anaesthesia and surgery are well established. However, their use has declined due to ethical considerations and increasing costs, and as research interest has moved towards genetics. Dogs are still used for some types of research, mainly in pharmacology and toxicology3, but also for studies in dentistry, surgery and radiation oncology. In addition, some breeds of dog develop spontaneous conditions which are useful models for human diseases, such as muscular dystrophy and cardiovascular diseases. Dogs are particularly suited to studies requiring close monitoring and frequent sampling. Over 400 breeds of dog are recognised, and purpose-bred laboratory dogs are usually beagles, which are of medium size and generally have a good temperament. Dogs for use in the laboratory in the UK must usually be sourced from a licensed breeder, and permanently marked for identification purposes; for example, with a tattoo in the ear flap, or with a microchip implanted between the shoulders.

Behaviour

Dog behaviour is increasingly understood, and is very complex. In the natural situation, dogs are thought to live like wolves in packs, tightly knit groups consisting of a breeding pair and offspring, in which the animals demonstrate a complex range of social behaviour. In this situation, dominance and aggression are rare: the breeding animals are dominant, and offspring disperse as they approach puberty. Leadership behaviour in younger pack members tends to be followed by dispersal[5]. Members of a pack cooperate in activities such as hunting. Dogs are crepuscular, being most active at dawn and dusk[6]. Therefore they may rest for much of the day and night.

In captivity, dogs can learn to exist in groups of unrelated animals without aggression. Properly socialised dogs can interact harmoniously with both unfamiliar dogs and humans. It is believed that dogs learn how to relate to other dogs and avoid conflict

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through play with their littermates at a young age. Dogs need to be socialised, to humans and other dogs, when they are young, and it is recommended that they be socialised and familiarised with conditions to be encountered during procedures between 4 and 20 weeks of age[7]. This social contact is vital for normal social development. Puppies must therefore be allowed to mix and play together from a young age, so they learn how to take part in complex social behaviour. It is believed that dogs cooperate with humans because they can integrate into a human 'pack'; again, early socialisation is essential if this is to occur. Well-socialised dogs trained using positive reinforcement training techniques will easy to handle and make good experimental subjects, as they will be only minimally stressed by handling and procedures. Purpose-bred laboratory dogs will usually have been raised in a pack environment with the opportunity to experience natural social behaviour, thus producing dogs that are likely to be temperamentally suited to the laboratory.

Five personality traits have been identified in dogs: playfulness, curiosity/ fearlessness, chase-proneness, sociability and aggressiveness[8]. The first four of these seem to be related, and describe a broad factor similar to the shyness–boldness axis seen in humans. This can be used to identify the social status of the animal at any time, and aid the selection of suitable dogs for particular studies or breeding. Social status affects behaviour and biochemical parameters such as cortisol levels, although it is not fixed and the social status of an individual animal will vary depending on the situation. Dogs show 'dominant' and 'subordinate' behaviour towards each other. However, many 'submissive' signals are essentially appeasement signals, and are designed to avoid aggression, but not necessarily in the context of a hierarchy[9]. Dogs with more confident/curious personalities may perform so-called dominant behaviours more often than other dogs5. Such behaviours include begging for food, standing over another dog, territory marking, holding the tail and ears erect, circling and sniffing and looking directly at other dogs, and growling. Behaviours shown by 'shy' or 'subordinate' dogs include adopting postures that are physically lower than other dogs, rolling over, lowering the tail, flattening the ears, dribbling urine, and freezing or fleeing from other dogs.

Dogs use many methods of communication, including behaviour, vocalisation and olfactory signals, and this should be considered when designing dog housing. People can learn the signals associated with greeting, nervousness, submission and aggression, making interaction with the animals easier[10].

Housing

Dogs are active animals, which need a lot of exercise and a complex environment. They can be kept in indoor pens, ideally with removable partitions, to allow linking of adjacent pens so the dogs can interact as a group, or be separated for procedures if required. Pens need to provide stimulation for the dogs, and may include platforms to allow the dogs to look out of the pens, secluded areas for privacy and toys such as chew sticks or balls[11]. There must be facilities for adequate exercise, either in the individual runs or in a communal exercise yard where dogs can go in pairs or groups and interact with each other and with the handler. Cages are unsuitable for dogs in the long term as there is little room for exercise or human contact. Environments that are inadequate lead to stereotypic behaviour and poor welfare.

The floors and walls of the accommodation area need to be of smooth, impervious material, which can withstand frequent cleaning. Partitions should be paw- and nose-proof to prevent aggressive encounters between neighbouring dogs and to reduce disease transmission, and flooring should be non-slip to prevent injuries to the dogs or handlers. Noise can be a significant problem in dog accommodation, and thought should be given to minimising this during construction. There must be a warm dry area for the dog to sleep in, containing a suitable bed and comfortable bedding material.

If the runs are large enough there will be no soiling of the bedding area, but faecal material should be removed from all areas daily. Puppies are not as clean as adults, and pens for nursing bitches may need to be cleaned two to three times daily. Scattering bedding material such as sawdust on the floors can help absorb waste and keep the pen and the dogs clean.

It is important that dogs in kennels have clear lines of sight to each other – this can reduce barking[12]. Males mark territory by cocking their legs, and frequent cleaning of the pen often results in frequent urination to re-mark the territory. Dogs can be encouraged to urinate in particular places by the selective use of odour eliminators. These will inhibit territory marking if used in areas which are required to be clean, such as the bed area and feeding area.

Dogs should be group or pair housed. They can become distressed if they are separated from their pack, although this can be overcome or decreased to an extent with training: young puppies can be habituated to periods alone from an early age. Therefore, single housing should be avoided unless it is essential for the study. Justification is required if dogs are to be singly housed for more than 4 h7, and dogs to be single-housed should first have been acclimatised to isolation. Singly housed animals should still be able to see and hear other dogs and should be handled at intervals throughout the day to reduce the development of stereotypic behaviour[12].

For more information about appropriate housing for dogs see references 13 and 14.

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Feeding

Dogs are carnivores, although they can eat a variety of foodstuffs including vegetable matter[15]. Dogs have few specific dietary needs, although they do require a dietary source of polyunsaturated fatty acids, deficiency of which can lead to skin problems3. Many commercial tinned and dry diets are available. Complete dry or semi-moist diets are often used and just need to be fed with water. These can be fed *ad libitum*, so the dog adopts a little-and-often pattern of feeding. Feeding infrequent, large meals of dry food predisposes to acute gastric dilatation and should be avoided. Sudden changes in diet may lead to digestive disturbances. To avoid competition for food in group-housed animals, several food stations should be available. It may also be beneficial to utilise toys into which food can be inserted, to provide stimulation and reduce boredom.

Dogs are very adaptable and deficiencies are rare. Diets usually contain over 50% carbohydrate, 19–22% protein and 5–10% fat, although dogs can survive on much lower protein levels. Feeding excess protein can lead to renal damage, but protein deficiency leads to reduced feed intake3. A 13 kg beagle needs 0.8 kg of canned food or 0.25 kg of dry food daily for maintenance, depending on the energy density of the diet. The ration should be increased by 30% for the last 3 weeks of gestation, and raised to three or four times the maintenance amount during lactation. For peak lactation, a high-energy food with a high calcium level should be fed, to prevent metabolic problems such as hypocalcaemia.

In addition to their maintenance diet it may be necessary to feed dogs additional materials aimed at reducing dental and periodontal disease. Chews and biscuits designed for this purpose are available.

Water

Water should be provided *ad libitum*. The average water requirement is 70–80 ml/kg/day, and active dogs will require more than this. An adult beagle therefore needs approximately 1 L daily, which will be provided by food and water. Dogs fed on dry diets will need more water than those on moist diets.

Environment

Dogs are very adaptable. They can cope with temperatures between 15 and 24°C, and lower temperatures are tolerated if there are no draughts and the dogs are in groups. Indoor pens are usually kept between 15 and 21°C. Extremes should be avoided. Neonatal dogs require higher temperatures (30–32°C) for the first 10 days: some of this is provided by the bitch but the air should be at least 26–28°C. By 4 weeks 24°C is adequate. Natural daylight is preferred, but if this is not possible a 12 h/12 h light/dark cycle is acceptable. A gradual increase or decrease in light intensity simulating dawn and dusk may be beneficial and avoid startling the dogs.

Breeding

Puberty is reached at 5–12 months of age. Bitches are monoestrous, and typically come into season twice yearly. Each season is followed by a period of anoestrus lasting 4–14 months. The season starts with pro-oestrus, characterised by a high oestrogen levels and a sanguinous vaginal discharge, which reduces as the bitch enters oestrus, after 6–10 days. The bitch is then receptive for 6–12 days, then she enters dioestrus, which is characterised by high progesterone levels and lasts about 60 days3. The bitch only permits mounting during oestrus, and the male and female ‘tie’ for 20–30 min during copulation, in which the male dismounts and turns to be rear to rear with the female. Ovulation is spontaneous and pseudopregnancy is common. Oestrus can occur at any time of the year, but is most common in the spring. Gestation lasts 59–67 days. Parturition (whelping) typically takes 3–6 h once the cervix is open. An average of six puppies is born, at intervals of up to 1 h. The bitch needs bedding material to provide comfort and aid thermoregulation in the pups.

To breed dogs in packs, one male is put with up to 12 bitches, and bitches are removed from the pack for whelping. Alternatively, bitches can be kept in pairs and moved in with the male 10 days after the onset of pro-oestrus for 5 days.

Growth

Pups are born blind and deaf and unable to stand. They will normally stand at about 10 days, and can walk by day 21.The eyes open at about 12 days, and ears open from 12–20 days. Puppies begin feeding immediately after birth. Puppies must get colostrum during the first 24 h, and are fed initially every 2–4 h. The bitch licks the puppies to stimulate respiration at first, then regularly licks the perineal area of each puppy to stimulate urination and defaecation for the first 3 weeks.

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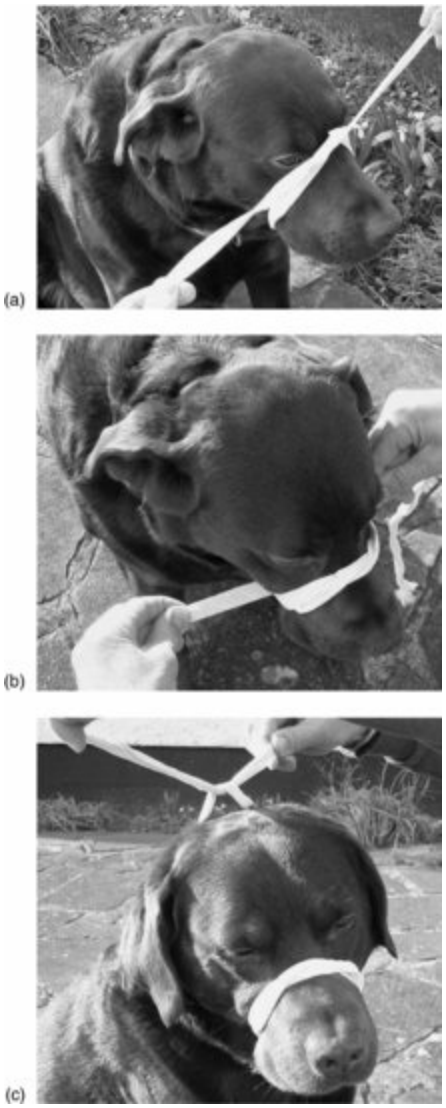
Puppies can start to eat solids from about 3 weeks, and may be weaned at 6 weeks. Growing puppies need to eat twice the maintenance level of an adult of similar size. Puppies should multiply their birth weight by a factor of 40 or 50 in their first year, and adult weight is reached at 9 months.

Handling

To facilitate procedures, it helps if the dog is submissive, so an understanding of dog behaviour can help establish a relationship with the dog where the handler is dominant. The dog should be approached quietly but confidently, talking to the dog to provide reassurance and avoid startling them. Lower yourself to the animal's level, which is less threatening, then offer the back of a hand for the animal to sniff. Make sure you can escape if necessary, and do not trap the dog in a corner. The lead and collar, or the scruff of the neck if there is no lead, can then be grasped in the other hand once the dog has accepted the person15.

To lift a dog, approach the dog from the left-hand side. Restrain the head with the left hand by either placing the arm under the neck and holding the dog's head against the handler's chest, or by holding its collar or scruff (gently), and lift the dog by placing the right arm over the body of the dog and taking its weight on its sternum. Alternatively, the dog may be lifted by placing one arm around the chest in front of the forelegs and the other behind the hindlegs, holding on to the outer legs. If a dog is nervous or aggressive, it may be muzzled, using a ready-made plastic or fabric muzzle, or with a tape (see Figure 12.1).

FIGURE 12.1 How to put a tape muzzle on a dog.



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Pain and stress recognition

Familiarity with the individual dog's normal behaviour allows identification of any changes brought about by the presence of pain. A dog in pain or distress may show physiological or behavioural signs (see Table 12.1). They may whimper or howl, and growl without provocation. They may bite and scratch at painful areas and may become more vicious or aggressive. The animal may guard a painful area by altering its normal behaviour to avoid moving it.

Table 12.1 Signs of pain and distress in dogs15.

Physiological signs
Increased or decreased respiratory rate, or panting
Pallor, cyanosis or jaundice
Poor skin and coat condition
Discharge from eyes, nose, urinary or genital tracts or ears
Increased or decreased body weight
Vomiting or diarrhoea
Increased or decreased appetite or water consumption
Increased or decreased urination
Shivering
Abnormal posture: hunched, tense or guarded abdomen
Behavioural signs
Dullness, depression or lethargy
Unresponsiveness
Increased aggression
Increased sleep time
Isolation from group or hiding
Vocalization
Restlessness

Common diseases and health monitoring

The major risks for disease entry in dog colonies are personnel or new arrivals. Disease entry can be minimised by providing quarantine facilities and serological screening for new arrivals, and by personnel adhering to strict entry requirements. New arrivals should be acquired from known sources with clean health records, effectively quarantined on arrival, and given a veterinary health check as soon as possible.

There should be a regular health monitoring and preventive care programme, including regular veterinary health checks, routine screening and investigation of any unusual occurrences and illnesses. Routine laboratory screening every 3 months is recommended[16]. Samples of blood, skin/hair and faeces should be analysed for a range of diseases. Most of the major diseases of dogs are preventable by regular vaccination: screening for these diseases in vaccinated dogs is not necessary. Other diseases can be prevented by adhering to strict entry requirements, good hygiene and by regular preventive treatments.

Dogs can be vaccinated against canine adenovirus, canine distemper, canine parainfluenza virus, canine parvovirus, *Bordetella bronchiseptica* and leptospirosis. They may also develop clinical disease caused by coronavirus, rotavirus or parasite infestations. Zoonotic diseases potentially carried by dogs include leptospirosis, rabies, *Salmonella*, *Campylobacter*, *Toxocara canis*, Lyme disease and brucellosis. Many of these can also affect research; for example, respiratory pathogens compromise studies involving anaesthesia and *Toxocara* affects toxicological pathology studies.

Biological data and useful reference data

See Table 12.2.

Table 12.2 Useful data: dog (beagle).

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Biological data		**Breeding data**	
Adult weight (kg)	7–17	Puberty (months)	5–12
Diploid number	78	Age to breed (years)	1–2
Daily food intake (g/kg)	18 (complete dry diet)	Gestation (days)	59–67 (average 63)
Daily water intake (ml*)	1200	Litter size	1–12 (usually 4–6)
Natural lifespan (years)	10–15 (larger breeds have shorter lifespans)	Birth weight (g)	250
		Weaning age (weeks)	4–6
Rectal temperature (°C)	37.9–39.9	Oestrous cycle	Monoestrous (oestrus interval of 7–8 months)
Heart rate/min	70–160		
Blood pressure (mmHg)			
Systole	100–178		
Diastole	52–104	Post-partum oestrus	No
Blood volume (ml/kg)	76–107		
Respiratory rate/min	20–40		
Tidal volume (ml)	251–432		
Haematological data		**Biochemical data**	
Red blood cells ($\times 10^6$/mm^3)	5.5–8.5	Serum protein (g/dl)	6–7.5
		Albumin (g/dl)	3–4
PCV (%)	37–55	Globulin (g/dl)	2.4–3.7
Hb (g/dl)	12–18	Glucose (mg/dl)	54–99
White blood cells ($\times 10^3$/mm^3)	6–17	Blood urea nitrogen (mmol/l)	3.5–7.5
Neutrophils (%)	60–70	Creatinine (μmol/l)	120
Lymphocytes (%)	12–30	Total bilirubin (μmol/l)	5.0
Eosinophils (%)	2–10	Cholesterol (mmol/l)	4–7
Monocytes (%)	3–10		
Basophils (%)	0–1		
Platelets ($\times 10^3$/mm^3)	200–900		

Hb, haemoglobin; PCV, packed cell volume.
*Variation with size and diet composition.

Anaesthesia

A period of at least 12 h starvation should precede anaesthesia in the dog. It is not necessary to withhold water. It is usual to induce anaesthesia by intravenous injection following suitable premedication, then to maintain anaesthesia by inhalation using isofluane and oxygen with or without nitrous oxide. Premedication is essential to alleviate any anxiety and ensure a smooth induction and recovery from anaesthesia. See Table 9.3c for doses for premedication, anaesthesia and analgesia in dogs.

Ferret

The ferret, *Mustela putorius furo*, is important as a laboratory animal because it is a carnivore that is small enough to be kept easily in the laboratory. There are two main varieties, the fitch ferret, which is buff with a black mask and points, and the albino. Both male and female ferrets show marked seasonal variations in the hair coat, and body weight fluctuates by up to 30–40%, as subcutaneous fat is laid down in the autumn and shed in the spring[17]. Male ferrets are called hobs, and females jills.

Domestic ferrets are believed to be a domesticated form of the wild European polecat. They appear to have been used by humans for at least 2500 years. References to ferret-like animals used for hunting have been found in ancient Greek and Roman records[17,18]. Ferrets have been used for hunting, and for the control of rodents and snakes. They can be trained to work on leads or lines and have been used to lay cables. They have also been bred for their fur, known as fitch. Ferrets are still used for hunting today but increasingly are kept simply as pets.

The domestic ferret belongs to the family Mustelidae which includes stoats, weasels, badgers and mink. All mustelids secrete a

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strong smelling musk from their anal glands, and ferrets are no exception. Their Latin name, *Mustela putorius furo*, translates as 'weasel-like smelly thief'.

Mustelids typically have sleek, flexible, elongated tubular bodies, with short legs and small rounded ears. These characteristics allow them to move freely and turn round in confined spaces.

Behaviour

Ferrets are domesticated animals, and are not generally found in the wild. Studies of feral ferrets and the European polecat suggest that they are largely solitary[19]. However, domestic ferrets are sociable and gregarious, and seem to benefit from being kept in compatible groups.

Ferrets are highly intelligent, lively and curious. They spend up to 75% of the day asleep, but the remainder of the time will be very active. They are more active at night[20]. They like to sleep in dark, enclosed areas. They are agile and like to explore and burrow, and they will make good use of three-dimensional environments containing playthings and multilevel perches. They are not frightened of humans or human environments18. Their curious nature means they are prone to escape through any hole large enough to get a head through. This can have tragic consequences for both the ferret and any rodents or birds housed in adjacent areas[21]. Housing design should take these factors into account.

Ferrets have an ill-deserved reputation for being aggressive. Although they have retained many of their natural behaviour patterns, they are more docile than polecats. They may bite if nervous, particularly if handled roughly, and may mistake a tentatively approaching hand for food, but otherwise they are usually friendly, particularly if handled frequently from an early age. Females with litters are protective and may also bite. Young ferrets may nip when first handled, but this abates with frequent handling.

Ferrets communicate by using musk glands, which are situated lateral to the anus. The secretion may also be expressed when excited or frightened, or during the breeding season. They also vocalise and produce a number of different sounds: they may hiss and chuckle when playing, and may scream if frightened or threatened.

Housing

Ferrets will demarcate a number of different areas in their accommodation: a sleeping area, an area for food storage, several escape holes and a latrine area. They prefer to urinate and defaecate in one or two latrine areas within the enclosure, often vertical surfaces, keeping the rest of the cage clean, and ferrets can be trained to use a litter box.

Groups of jills without litters, young animals and castrated males (hobbles) can be kept together, although group housing is not advisable for adult hobs, jills with litters and females that are in oestrus or have been mated. Young ferrets will readily play together.

Housing for ferrets needs to be particularly secure. Their slender bodies and extreme flexibility allow them to exploit the smallest gaps, and they are notorious escapers. Ferrets prefer solid floors with bedding such as sawdust or shavings rather than grid floors.

Plastic tubes, boxes and paper bags will add to the richness of the environment, and the animals will readily explore and play with them. Ferrets will make good use of three dimensional space if given the opportunity (see Figure 12.2). However, ferrets are also prone to chewing and eating such objects, so they should be chosen with care to avoid intestinal foreign bodies. For breeding females, nest boxes should be provided to afford security and warmth for kits.

FIGURE 12.2 Ferrets will make good use of three-dimensional environments.

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Feeding

Ferrets are obligate carnivores. The nutritional requirements of ferrets have not been studied extensively. They have little ability to digest fibre, and they eat to energy requirements. The gut transit time is short (3–4 h), so they need highly digestible diets. They need a diet high in protein (30–40%) and fat (18–20%), with low fibre and carbohydrate. Dry diets are often soaked for ferrets and fed as a stiff paste17,18,21.

Water

Water is given *ad libitum* from bottles or cups. Galvanised water and food bowls should not be used for ferrets as they are susceptible to zinc toxicity.

Environment

Ferrets can tolerate a wide range of temperatures. The temperature should be between 15 and 24°C, and the humidity 45–65%. Ferrets can tolerate low temperatures, but are susceptible to heat exhaustion above 30°C because they have poorly developed sweat glands. Unweaned young should be kept above 15°C7. Good ventilation is required. A 12 h light cycle is usual, but changes in the light cycle can be used to manipulate the breeding cycle.

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Breeding

Puberty occurs at 9–12 months, in the spring following birth. Breeding is seasonal, and the breeding season is determined by photoperiod. Males come into season as the day shortens, whereas females respond to increasing day length. In the northern hemisphere males are therefore in breeding condition between December and July, and females between March and September. The males need to come into season before the females to allow for sperm maturation. Ferrets are usually mated at the start of the season, and have a litter in late spring or early summer. Lactation then prevents a return to oestrus, and once the offspring are weaned the days are getting shorter so fertility is reduced. Rarely, a second litter may be born in late summer. Year-round breeding can be achieved by manipulation of the light cycle, by having summer (e.g. 16 h light/8 h dark) and winter (8 h light/16 h dark) rooms, or 'northern' and 'southern' hemisphere rooms. Artificial lighting can induce oestrus early and prolong the season. Females are induced ovulators, with no obvious breeding cycle. The vulva of the female enlarges to signal oestrus at the beginning of the breeding season, and if not mated ovulation does not occur. In this event, persistent oestrus may develop, in which the prolonged high levels of oestrogen can lead to bone marrow depression. To prevent persistent oestrus it is advisable to spay female ferrets that are not to be bred17.

Jills should always be checked prior to mating to make sure they are in good condition. Inadequate diets may result in failure to conceive or lactation failure, particularly if the diet lacking in animal protein17. Oestrous females should be taken to the male for mating about 14 days after the onset of vulval swelling, and left for 2 days. The male will grasp the female by the scruff of the neck and drag her around the cage. Mating can take several hours and may be rough and noisy. Coitus results in ovulation after 30–35 h. If unfertilised, pseudopregnancy may develop. After mating, jills should be housed away from stud males, and moved into their littering cages about 2–3 weeks prior to parturition. Otherwise, gestation lasts around 42 days, and seven or eight kits are born. Jills often look scruffy during gestation, and may need additional nutrition.

Dystocia (difficult birth) is fairly common in ferrets, and neonatal mortality can reach 8–10%21. Weaning occurs at 6–7 weeks. If bred early in the year, a female may have two litters in a year. Breeding performance declines after approximately 3 years of age in males, or after three or four litters in females.

Pregnant females can become aggressive as gestation progresses, and are very protective of their young, so should be handled with care.

Growth

All kits have white hair regardless of their eventual coat colour. They weigh 6–12 g at birth. They have voracious appetites, attaching to the nipples immediately after birth if possible, and they develop rapidly, doubling their birth weight in 5 days. They are active from about 14–21 days, they begin eating solid food by 2–3 weeks, and can be weaned at 6–8 weeks. They are born blind and deaf: the eyes open at 28–34 days and they hear from 32 days. Adult weight is reached by 4 months of age, and males are twice the size of females.

Handling

Although friendly, ferrets will bite if startled. They have relatively poor eyesight, and will bite a tentatively proffered finger, thinking it is a prey object. The important rules when handling ferrets are (1) be positive, calm and confident, (2) know what you are doing, (3) keep movements smooth and decisive and (4) use your voice as well as your hands[22]. This way, the ferret will not be startled. Well-handled ferrets may be picked up by simply slipping a hand under the animal just behind the forelegs, supporting the rump with the other hand. Alternatively, approach the ferret by distracting him with a cloth or sleeve held in one hand, then grasp him with the other hand using an over the shoulder grip, or with the thumb and forefinger encircling the neck with the other fingers under the forelimbs (see Figure 12.3). Lift the animal up and support the hind quarters with the other hand. When dealing with unknown animals gloves may be advised. Ferrets will normally wriggle when first picked up, but should soon calm down. See also Figure 7.6 for jugular venepuncture in the ferret.

FIGURE 12.3 Handling a ferret.

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Pain and stress recognition

Ferrets are stoic animals and may not show clinical signs until a disease is advanced. Ferrets should be active and explore vigorously once they are awake. A sick ferret will be lethargic and disinterested in its environment. There may be reduced food intake and weight loss. If approached they may offer little resistance to handling, or may react with aggression and try to bite. The eyes may become puffy and half closed, and the coat will be ungroomed and take on a scruffy appearance.

Common diseases and health monitoring

Zoonotic diseases

Ferrets may carry a number of enteric pathogens, such as *Salmonella* and *Campylobacter*, so good hygiene is essential.

Subclinical and clinical diseases

Ferrets are prone to several viral diseases, the most significant of which are influenza, canine distemper, Aleutian disease and ferret coronavirus.

Influenza can spread from people to ferrets, and vice versa. The disease in adult ferrets is usually mild, but it can cause mortality in kits. Care should be taken to wear a mask when dealing with ferrets if you have a respiratory infection.

Canine distemper virus can cause an acute disease in ferrets with nearly 100% mortality17. There is no treatment, and affected animals should be euthanased. Animals entering the colony should be of known health status and should be tested serologically to avoid bringing the disease in, or a vaccination policy should be employed.

Aleutian disease was originally a disease of mink, but has been found in pet and working ferrets[23]. It can cause many clinical signs, depending on the organs affected. It can be detected by serology on a small blood sample, and positive animals should be removed from the colony. There is no treatment or vaccine available.

Ferret coronavirus occurs in two forms, an intestinal form which causes green diarrhoea, and a systemic form. The intestinal

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form is self-limiting but animals may take several months to recover fully.

Jills are induced ovulators, and if not bred will remain in season for a prolonged period, lading to hyperoestrogenism. High levels of oestrogen produced cause bone marrow depression. If animals remain in oestrus for more than 2 months they can develop life-threatening anaemia and haemorrhage17,20,21. Females should not remain in heat for longer than 1 month. This condition can be prevented by either spaying non-breeding females, changing the lighting to the winter cycle, hormone treatment or housing with a vasectomised male to induce ovulation.

Diseases affecting research

Many diseases can potentially affect research. Ferrets are often used in auditory research, and may suffer from ear mite (*Otodectes cynotis*) infestation. Aleutian disease virus can interfere with neurological research and any respiratory infection will increase the chances of anaesthetic deaths.

Ferrets may develop gastric ulcers caused by *Helicobacter mustelae*17. Clinical disease is rare but under stress it can lead to ulceration and death. Clinical signs include anorexia, weight loss, vomiting, ptyalism, tooth grinding and black stools.

Biological data and useful reference data

See Table 12.3.

Table 12.3 Useful data: ferret[17,24].

Biological data		**Breeding data**	
Adult weight*	Male 1–2 kg Female 600–900 g	Puberty (months)	9–12
		Age to breed (days)	
Food intake (g)†	50–75	Male	365
Water intake (ml)	75–100	Female	275
Natural lifespan (years)	5–11	Gestation (days)	38–44 (average 42)
Rectal temperature (°C)	37.8–40		
Heart rate/min	250	Litter size	1–14 (average 8)
Blood pressure (mmHg)			
Systole	110–140	Birth weight (g)	6–12
Diastole	31–35	Weaning age (weeks)	6–8
Blood volume (ml/kg)	45–70	Oestrous cycle	Induced ovulator
Respiratory rate/min	33–36		
Diploid number	40		
Haematological data		**Biochemical data**	
Red blood cells ($\times 10^6$/mm^3)	6.8–12.2	Serum protein (g/dl)	3.5–7.4
		Albumin (g/dl)	2.6–3.8
PCV (%)	42–61	Globulin (g/dl)	2.5–4.8
Hb (g/dl)	12–18	Glucose (mg/dl)	94–207
White blood cells ($\times 10^3$/mm^3)	4–19	Blood urea nitrogen (mg/dl)	10–45
		Creatinine (mg/dl)	0.2–0.9
Neutrophils (%)	11–84	Total bilirubin (mg/dl)	0–0.4
Lymphocytes (%)	12–69	Cholesterol (mg/dl)	64–296
Eosinophils (%)	0–7		
Monocytes (%)	0–8		
Basophils (%)	0–2		
Platelets ($\times 10^3$/mm^3)	297–910		

Hb, haemoglobin; PCV, packed cell volume.
*Both sexes show a periodic weight fluctuation of 30–40%. Fat is laid down in autumn and lost in winter.
†Dry carnivore pelleted diet. This can be fed soaked in hot water to form a paste.

Anaesthesia

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Before inducing anaesthesia it is important to weigh the animal, since a thick coat may lead to overestimation of body weight, and the seasonal weight fluctuation may mean that the animal's weight has changed significantly between weighings. Injectable agents should be dosed by weight, but in the winter ferrets accumulate fat and may require relatively more anaesthetic. Ferrets vomit readily, and should be fasted for 3–4 h prior to induction of anaesthesia to minimise the risk of vomiting. Longer periods of fasting are not necessary, since the gut transit time is short, and may lead to hypoglycaemia.

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Chapter 13

Primates

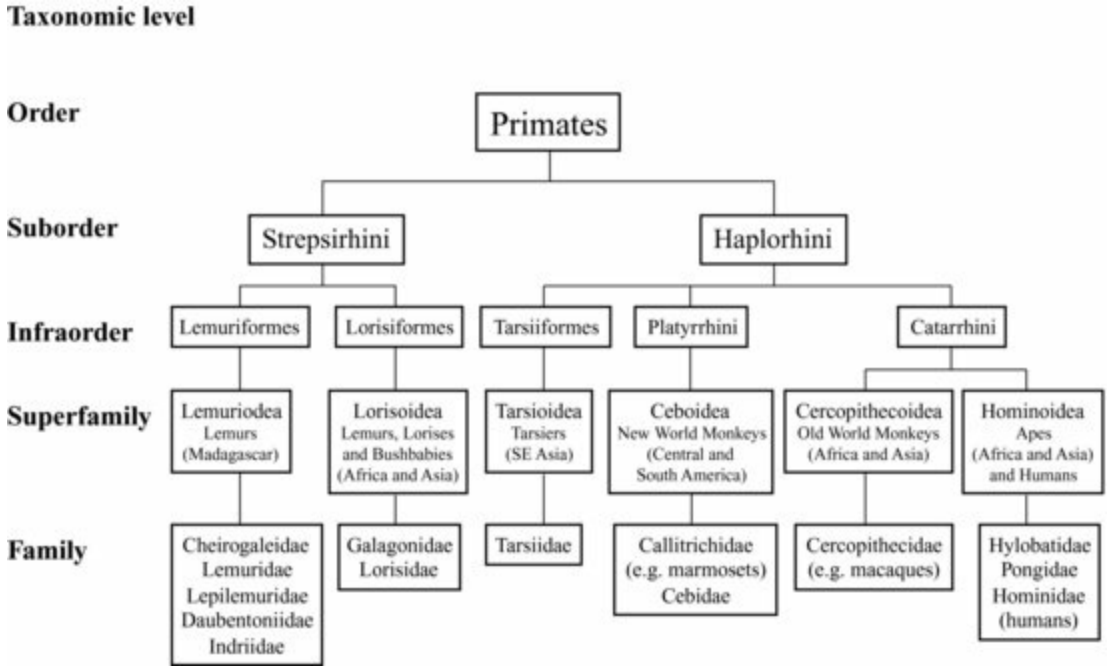
Use of Primates in Research

Primates should only be used in research programmes where there is particular justification and where it can be demonstrated that the benefits to society outweigh the harms inflicted on the animals that are used[1,2]. While this principle applies to the use of all animals used in research, there is a particular societal concern and uncertainty over the acceptability of using primates in research, principally because of their evolutionary proximity to human beings. This has led to the view that primates may have a greater capacity for suffering than other animals because of their more developed cognitive abilities[3]. It is therefore particularly necessary when considering the use of primates to consider the ethical issues as well as the practical ones (see Chapter 2).

The total number of primates used annually in research worldwide is estimated at between 100 000 and 200 000, with 64.7% involving Old World monkeys[4]. Most (up to 70%) are used in regulatory toxicology. The most common research areas for which primates are used are infectious diseases (including HIV/AIDS) at 26%, neuroscience 19%, biochemistry 12% and pharmacology/physiology 11%, in addition to their use in regulatory toxicology. The use of non-human primates in research in the UK has been comprehensively reviewed by Wetherall1 and Bateson2.

The primates can be defined according to anatomical features[5,6]. A simplified classification of living primates is shown in Figure 13.1[7]. The term 'Old World monkeys' indicates a geographical origin from Asia and Africa, as opposed to the 'New World monkeys' which originate from the Americas. New World and Old World non-human primates should be housed separately as latent infections in one group can cause serious clinical disease in others.

FIGURE 13.1 A simplified classification of primates. Adapted from Wolfensohn and Honess7.



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New World Monkeys

New World monkeys originate from tropical rain forests in Central and South America, where they are exclusively arboreal. Their native environment may be hot and humid, and the animals are exposed to sunlight. Ultraviolet light should be supplied if possible, but if not then vitamin D_3 must be added to the diet. There are many differences between outwardly similar species of New World monkeys, and the different regimes encountered in captivity are likely to affect their responses in different ways. Animals may thrive in one captive environment, but exhibit poor reproductive capacity or ill health in another very similar environment, or even if moved to a different position in the same room. Social and environmental enrichment appear to play a particularly important role in maintaining the health of these small primates. For more details on marmosets and tamarins see Buchanan-Smith[8], on squirrel monkeys see Williams et al.[9] and on capuchins see Anderson and Visalberghi[10].

Marmoset

The commonest New World species used in research is the common marmoset, *Callithrix jacchus*. Marmosets are small and relatively easy to keep in the laboratory. For biological data see Table 13.1.

Table 13.1 Useful data: marmoset

Biological data		**Breeding data**	
Adult weight (g)	300–400	Puberty (months)	8
Diploid number	46	Age to breed (years)	1.5–2
Food intake	20 g daily (New World monkey pelleted diet)	Gestation (days)	140–148 (average 145)
		Litter size	Usually dizygotic twins (can be 1–4 offspring)
Lifespan (years)	10–16		
Rectal temperature (°C)*	38.6 (day), 36.3 (night)	Birth weight (grams)	25–35
		Weaning age†	6 weeks to 6 months.
Heart rate/min*	230–312		
Mean arterial pressure (mmHg)*	65–100 (day), 50–95 (night)	Oestrous cycle (days)	14–28 (few overt signs of oestrus)
		Comments	Interbirth interval 154–178 days
Blood volume (ml/kg)	70		
Haematological data		**Biochemical data**	
Red blood cells ($\times 10^6/mm^3$)	5.7–6.95	Serum protein (g/dl)	6.6–7.1
		Albumin (g/dl)	3.8
PCV (%)	45–52	Globulin (g/dl)	2.7–3.9
Hb (g/dl)	14.9–17	Glucose (mg/dl)	126–228
White blood cells ($\times 10^3/mm^3$)	7.3–12.8	Blood urea nitrogen (mg/dl)	51.8
Neutrophils (%)	26–62	Creatinine (mg/dl)	0.9–1.2
Lymphocytes (%)	30–67	Total bilirubin (mg/dl)	0.4–0.6
Eosinophils (%)	0.6–4.2		
Monocytes (%)	0.4–5		
Basophils (%)	0.1–1.1		
Platelets ($\times 10^3/mm^3$)	490		

Hb, haemoglobin; PCV, packed cell volume.
*Marked diurnal changes: heart rate, arterial pressure, activity and body temperature all fall at night.
†Young can suckle for longer, particularly if stressed.

Behaviour

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Wild marmosets live in family groups of three to eight animals or more, with a well-developed social structure. Males and females pair for life, and both parents care for the young. Individuals show a great deal of interaction, including play and mutual grooming. There is marked diurnal variation in their behaviour, and they are much less active at night.

Housing

Caging for marmosets needs to reflect their behavioural needs. Since they are arboreal they climb well and if startled they flee upwards, not along the ground, so cages for these species should be tall and narrow to reflect this. The stocking density within the room must be kept low, since high stocking densities and overcrowding are associated with increased levels of disease. Marmosets are usually housed in family groups composed of an adult pair and offspring. They are agile and like to climb, and so benefit from branches or other suitable perches within the cage. They will walk along branches on all four feet and use their tails to balance.

Feeding

Marmosets will eat freshly milled New World monkey diet, but benefit from supplements such as pieces of fruit or malt loaf. Diets for New World monkeys usually contain 20–25% protein, and protein should be fed at 3 g/kg of body weight daily. New World monkeys require vitamin D_3, which they normally produce during exposure to ultraviolet light. In the laboratory it should be added at 1–2.5 i.u./g of diet. A deficiency in it will predispose to pathological lesions of the bones. Deficiencies in vitamin E, selenium and protein may lead to wasting marmoset syndrome. Adult marmosets eat about 20 g of pelleted diet daily. Water is supplied *ad libitum*, using bottles or automatic systems. With bottles, vitamins as described above can be added to the water. Drilled wood filled with gum provides good enrichment. Food hoppers and water bottles can be cleaned twice weekly.

Breeding

Marmosets, as all New World monkeys, have oestrous cycles, whereas Old World monkeys have menstrual cycles. Sexual maturity is reached at 14 months; the female is polyoestrous, having cycles every 14–28 days. However, no overt signs of oestrus are seen. If they are kept in large groups with more than one female, only the dominant female will breed. Mating results in gestation lasting 140 days, and twins are usually carried. The fetuses share a placenta with vascular anastomoses, so they share their blood supply and are natural chimeras. After birth, the young are normally weaned at 6 months and stay with their parents until sexual maturity. Typically the males do much of the infant carrying. Females are mated again shortly after parturition and the interbirth interval is 154–178 days. With improved nutrition and health of marmoset colonies, between 20 and 52% of pregnancies may result in triplets. Left alone, the weakest one will drop off at around the third day and die, which raises ethical and welfare issues. The alternatives are rotational feeding, hand rearing or removing one infant for euthanasia. Each of these has certain disadvantages and the management method selected to deal with these extra offspring will depend on the individual circumstances of the colony.

Growth

Marmosets reach their maximum body weight by 20 months of age.

Old World Monkeys

The Old World monkeys all lack prehensile tails and have some degree of callus over the ischial tuberosities. Many have cheek pouches, and all have a narrow nasal septum and opposable thumbs. The Old World monkeys most commonly used in research are *Macaca mulatta* (rhesus macaque), *Macaca fascicularis* (long tailed, crab-eating or cynomolgus macaque), *Chlorocebus* sp. (formerly *Cercopithecus* sp.) (sometimes called the African green monkey or the vervet monkey) and *Papio* species (baboons). For biological data for the macaques see Table 13.2. The macaque genus has a wide natural range, extending from Morocco and Gibraltar through the Indian subcontinent up through China and Japan and down through the Philippines, Java and Sumatra. This range includes a wide variation in environmental temperatures and diets, and the macaques have adapted to a variety of ecological niches. This is reflected in normal variations in patterns of behaviour and interactions with humans, for example foraging around urban environments[11] to foraging in remote areas.

Table 13.2 Useful data: macaque

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Biological data	Rhesus macaque (*Macaca mulatta*)	Cynomolgus macaque (*Macaca fascicularis*)
Adult weight (kg)	Male 6–11, female 4–9	Male 4–8, female 2–6
Diploid number	42	42
Food intake	420 kJ/kg for maintenance, 525–630 kJ/kg for production, 840 kJ/kg for neonates.	
Lifespan (years)	20–30	15–25
Temperature (°C)	36–40	37–40
Heart rate/min	120–180*	240
Blood pressure systole (mmHg)	125	As rhesus
Blood pressure diastole (mmHg)	75	As rhesus
Blood volume (ml/kg)	55–80	50–96
Respiratory rate/min	32–50*	
Breeding data		
Age at puberty (years)	Male 3–4, female 2–3	3–4
Age to breed (years)	Male 4–5, female 3–5	4–5
Gestation (days)	146–180 (average 164)	153–179 (average 167)
Litter size	1	1
Birth weight (kg)	0.4–0.55	0.33–0.35
Weaning age (months)	10–14, can hand rear from birth	14–18
Breeding cycle	Menstrual cycle 28 days	Menstrual cycle 31 days
Comments	Seasonal breeding, September–January	Non-seasonal breeding

Haematological data		Biochemical data	
Red blood cells ($\times 10^6/mm^3$)	3.56–6.95	Serum protein (g/dl)	4.9–9.3
		Albumin (g/dl)	2.8–5.2
PCV (%)	26–48	Globulin (g/dl)	1.2–5.8
Hb (g/dl)	8.8–16.5	Glucose (mg/dl)	46–178
White blood cells ($\times 10^3/mm^3$)	2.5–26.7	Blood urea nitrogen (mg/dl)	8–40
Neutrophils (%)	5–88	Creatinine (mg/dl)	0.1–2.8
Lymphocytes (%)	8–92	Total bilirubin (mg/dl)	0.1–2
Eosinophils (%)	0–14	Cholesterol (mg/dl)	108–263
Monocytes (%)	0–11		
Basophils (%)	0–6		
Platelets ($\times 10^3/mm^3$)	109–597		

*Values determined under sedation.

Sources, supply and transportation

In Europe the facilities for breeding macaques for research use do not yet supply adequate numbers to match demand. A substantial number of macaques are, therefore, imported into Europe from countries of origin. Since macaques come from a wide range of natural habitats, the genetic backgrounds and responses to experimental procedures will vary for macaques from different populations[12]. Evidence suggests that when primates are subjected to a stressful event, such as transportation, signs of physiological stress are markedly reduced when in the presence of other individuals[13]. Juvenile animals may be shipped in pairs within the transport container and the compatibility of paired animals can be ensured by studying the interactions of the animals in their social groups prior to being transported; animals which show affiliation can be assigned to travel together. This approach should extend beyond the transport phase and efforts should be made to ensure that groups which will be housed together at their

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destination are socially stable and, where possible, from the same breeding group. In this way relationships and social status will have already been established in natal groups, reducing the likelihood and intensity of conflict. This makes future veterinary and behavioural management of the animals easier.

Communication between shipper and recipient should ensure the animals' favoured and acceptable foods can be available on arrival. A change in diet can affect the gut flora and will add to the animals' stress. A gradual change to a new diet over 4–5 days will reduce the incidence of diarrhoea. There may also be disease risks associated with the transportation of primates, both between the animals and handling staff and between consignments of animals, particularly if the health status of the animals is unknown.

Breeding

There are several types of captive breeding systems for primates, including free-ranging island colonies, semi-free-ranging corral colonies and enclosure or pen pairs or harems. A description of each is provided in reference 14. For further details on breeding see Wolfensohn[15] and Kirkwood and Stathatos[16].

In female rhesus monkeys puberty occurs at 2–3 years and 3–4 years in males. For cynomolgus monkeys puberty occurs at 3–4 years in both sexes. Rhesus monkeys have a menstrual cycle lasting 28 days, and breed seasonally from September to January. Cynomolgus monkeys are non-seasonal breeders, with a menstrual cycle of 31 days. Old World monkeys have sexual skin, which may swell and change colour to indicate the animal's readiness to breed. Ovulation in macaques is indicated by changes in the female's behaviour, olfactory cues and swelling and reddening of the perineal skin. The size and colour of the sexual skin indicates the occurrence of ovulation, and may also be associated with attractiveness in the context of mate choice, both of females to males and vice versa (e.g. in rhesus macaques[17]). In females the anogenital region is frequently the primary site of sexual skin, but it may occur in other areas, such as the side of the thigh in rhesus macaques. Sexual skin in male primates may also be dramatic and brightly coloured (e.g. the blue of the scrotum of the vervet monkey, *Chlorocebus aethiops*). In addition to visual signals of reproductive state, communication via auditory and olfactory routes is extremely important in many primate species. Gestation lasts 164 days in the rhesus and 167 days in the cynomolgus; and one infant is usually born. Dependence on the mother for nutrition gradually wanes between 3 and 6 months of age and the infant becomes independent for foraging and travelling by about 1 year of age. However, psychological support and protective intervention from the mother will extend well into the juvenile years.

The mother will terminate suckling at a point determined by the metabolic independence of the offspring, this being related to the offspring's body weight at birth and at weaning: the ‘metabolic weaning weight’. Natural weaning is not an abrupt event but a process lasting several months. It is during this time, and shortly after, that the young begin to learn from their mother and to refine food-selection, -acquisition and -processing skills[18,19]. After the first year, juvenile animals, especially males, become more and more involved in peer-group activities.

Opinions differ on the ideal time for separating offspring from their mothers[20,21,22,23]. However, if young monkeys are reared with an inappropriate social background they will show indicators of poor welfare such as a restricted repertoire of behaviours, an abnormal activity pattern, inadequate social behaviour and/or abnormal behaviours such as self-injury. Therefore it is recommended that weaning (meaning the physical separation from the mother) should not occur until after 12 months of age. Tracking the performance of animals during training within both the breeding facility and the experimental facility will help to identify individuals that produce offspring that are more likely to be easily trainable and to cooperate with research protocols. This will contribute, when coupled with the highest welfare standards in housing and husbandry, to a reduction in the stress of primates maintained for research.

Housing

The physical environment in which captive primates are housed contributes significantly to the physical and psychological health of the animals. Replication of the natural environment in the captive situation as far as possible ensures that the animals' health is maximized. Otherwise, although there may be plentiful provision of food and effective disease control, there may be a reduction in activity leading to boredom and obesity with resultant poor health.

Existing recommendations for primate housing are generally based solely on the weight of an animal without reference to linear dimensions, and the sizes of cages are sometimes too small to meet the behavioural needs of the animals, providing neither adequate space for exercise nor room for environmental enrichment[24]. The assumption is made that heavier individuals require more space than lighter ones, although in practice light, young animals are usually more active than the heavier adults7. Rather

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than basing cage size on parameters such as body weight or crown–rump length it is better to use performance standards which assess the ability of the animal to express its species-typical behaviours and which will vary depending on species, age, sex, individual temperament, group composition and dynamics[25]. It is important to appreciate that most recommendations for cage sizes represent *minimum* dimensions for animals of all ages. The duration of stay of the animal is also relevant. Where primates are to be kept for a period of years the necessity for housing them in an environment that satisfies their needs is even more important than for those animals that will only stay for a short while. There is an ethical obligation to house and maintain animals used in research in the best conditions that can be provided. Establishments wishing to demonstrate best practice should endeavour to produce conditions for their animals which provide key safe features of a naturalistic environment that will contribute to encouraging a range and frequency of behaviours as close to the natural situation as possible. So, as 'best practice' evolves it is necessary to continually re-evaluate whether the facilities and their operation and management can be improved.

Visual barriers are important in group-housing and multiple escape routes provide opportunities to avoid attacks and also prevent dominant individuals from restricting access of subordinates to other parts of the cage[26]. Where individuals may need to be treated individually, as in some toxicology experiments, areas with a partition to allow separation or varying degrees of social contact is required. There should flexibility of use incorporated the design of the housing.

Environmental enrichment is essential for captive non-human primates to meet their ethological and psychological needs[27]. It should provide the animals with the opportunity to carry out a sufficiently varied daily programme of activity. The actual enrichment provided will vary, but there is now a large literature offering a wide variety of options. The effectiveness of many is reviewed in Honess and Marin25. Opportunities for the animals to exercise a full locomotory repertoire should be provided in the living area. To allow this, the cage must be sufficiently large and contain adequate furnishings. Cage furniture itself can provide opportunities for a wide repertoire of behaviour. Perches, ladders, swings, plastic chains, car tyres, etc. are all of value, and allow the animals to utilise the three-dimensional space and have plenty of places to sit without having to squabble. This allows a social hierarchy to develop with less aggressive encounters.

Foraging is a natural and desirable behaviour which can occupy a significant period of time, reducing boredom and the incidence of stereotypic behaviours. Therefore, a substrate such as woodchips, hemp or shavings should be provided in which forage can be scattered. Hay, straw or other material such as shredded paper may be provided for environmental enrichment. Devices to encourage foraging (such as puzzle feeders) have also proved effective. Provision of foraging opportunities in an adequate amount of space is a way of offering a socially sanctioned method of establishing the hierarchy that does not result in significant wounding and injury. Some simple and practical methods include the use of wire grids over food hoppers, ice cubes of frozen juice, frozen grapes or melon cubes, food on top of the enclosure to encourage animals to forage upwards through the mesh, wrapping or boxing of treats, empty diet bags or cardboard boxes, and foraging boxes full of hay or sawdust[28]. Novelty is important so the items should be changed frequently to avoid habituation and a programme of rotation of items of environmental enrichment should be recorded. Reducing the incidence of aggressive encounters by resorting to long-term single housing of animals is not acceptable; the environment and the group composition need to be actively managed to ensure maintenance of social stability. A key component in a successful strategy is to start with the right compatible grouping, which may require coordination and effective communication with the source breeding colony which establishes the groups at weaning. Training animals to cooperate with carers and experimenters also helps to enrich the animal's life and reduces handling stress[29]. It is vital to remember that the animal's principal purpose is for a scientific procedure and the animal model must be valid in terms of normality and reproducibility. The environmental enrichment programme should be reviewed to consider any effect (positive or negative) on the quality of the scientific data generated.

Physical environment

The environmental sensitivity of primates varies considerably between species, some being more tolerant of environmental change than others. In the wild, rhesus macaques (*M. mulatta*) occur across a wide range of altitudes from sea level to 3000 m[30], indicating a tolerance of a substantial temperature range. Generally the temperature that will be comfortable for staff will also be optimal for the animals. Relative humidity levels should be maintained between 40 and 70%, but extensive daily washing, such as pressure-hosing, may result in surfaces which remain permanently wet, resulting in elevated humidity levels and also presenting a potential health risk to the animals as many pathogens remain viable in a warm, wet environment. Under certain experimental regimes and containment levels it may be important to ensure negative air pressure in the animal rooms.

Where access to daylight is not available a lighting regime of 12 h light/12 h dark is typically provided, in which case efforts should be made to replicate dawn and dusk gradations in light intensity. In the wild, primates rely on the ultraviolet radiation in daylight to produce vitamin D_3. Care should be taken to ensure that artificial light is provided using daylight spectrum tubes or bulbs, rather than standard fluorescent lighting. This is of particular importance where there may be a familial predisposition to

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metabolic bone disease or a deficit in dietary vitamin D_3[31]. Care should be taken in the positioning and intensity of artificial lighting in interior accommodation to avoid reflective glare off polished metal surfaces.

High noise levels may intimidate animals and have a negative effect on their psychological well-being. Primates are highly social animals and one of their primary modes of communication is vocal. Dominance can be asserted through vocalisations and therefore care needs to be taken in placement of overly assertive animals in proximity to those that may be negatively affected by vocal bullying. If cages are constructed using metal sheeting and heavy meshing with metal furniture, such as swings, noise production can be substantial. The use of materials such as wood will help reduce noise, as will flooring covered with sound dampening forage substrate such as wood chips. People working around the primates can generate considerable noise. It may be reassuring for the animals to hear the voices of familiar care staff but not all staff are viewed as friendly by the animals and their voices may, particularly when loud, produce a response suggesting stress or anxiety. Staff should be encouraged to keep voices down and to work around the animals in a way that reduces unnecessary loud noises, such as avoidance of slamming doors or crashing trolleys against walls.

Laboratory primates are often kept in cages or rooms, but some larger species may be kept in outside pens or corrals, with free access to a heated indoor area. There is a perception that there is a welfare advantage to all primates associated with the provision of external accommodation. However, it is the quality of the accommodation and the handling of the animals which matters most in terms of animal welfare and outdoor accommodation is not necessarily in itself a benefit. External accommodation carries with it risks from exposure to wildlife and disease transmission. The facility must be fit for purpose but there should be no underlying assumption that outside living areas are somehow automatically better[32]. A well-designed facility is critical to be able to deliver a successful animal care programme and behavioural management.

Husbandry regimes that may be more labour-intensive but have a reduced impact on the animals should be used. These will have other benefits; for example, reduced aggression and fewer traumatic injuries, and easier training producing a more valid research model.

Feeding/watering

Food

In the wild many primates choose to spend a substantial amount of their time foraging, and anything that may restrict this should be regarded as potentially detrimental to their welfare. It is better to provide small portions of easily accessible pelleted food with an unpredictable schedule, providing the remainder as forage to satisfy the animal's behavioural needs, rather than to provide the quantity required to satisfy the daily nutritional requirements in just one feed. Tables of the nutrient contents of foods commonly used to feed primates are published by the National Research Council[33].

An overview of energy requirements and how these are calculated is given in chapter 4 of Wolfensohn and Honess7. This energy requirement is met by a combination of commercial primate diet, a selection of fruit and vegetables and forage mix, but variety is essential and when adding any supplements such as fruit, care must be taken not to upset the nutrient balance. Amounts fed will also need to be adjusted according to levels of activity and weight gain/loss must be evaluated regularly.

Supplements are generally given for environmental enrichment or as part of positive reinforcement training, rather then primarily for nutrition but may have the undesired effect of distorting the balance of the nutrition provided. It is therefore important to use items that are nutritionally complete or which are high in moisture and low in calories – such as fresh fruit and vegetables – rather than offering energy-dense but nutritionally incomplete foods such as nuts and raisins. Since fresh fruit and vegetables are 80–90% moisture, if these make up less than 40% of the wet weight of the diet they will be providing less than 10% of the total dietary dry matter and therefore will minimally distort the nutrient balance.

Animals should be fed according to their individual needs, and these will depend on species, age, sex, physiological and reproductive status, health and environmental conditions. Growth can be measured in various ways such as body weight, crown–rump length, limb length or head circumference. The linear measurements are not distorted by accumulations of body fat in the way that body weight may be. Growth rate will vary depending on genetic background, maternal nutrition in pregnancy and lactation, availability of supplementary weaning foods and rearing practices. Both sexes show an adolescent growth spurt, particularly males.

Water

The amount of water drunk to satisfy physiological needs will depend on many factors such as the water and electrolyte content

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of the food eaten, the ambient temperature and humidity, and activity level of the animal. The mean total water intake in cynomolgus monkeys has been found to be 76±35 ml/kg per day for males and 100±51 ml/kg per day for females, of which drinking water comprised 50±33 ml/kg per day for males and 49±48 ml/kg per day for females, with other water being derived from food[34]. Even moderate restriction of water availability will reduce food consumption.

Health Monitoring, Quarantine and Common Diseases

When monkeys are brought into a facility basic information about them is necessary in order to be able to design the quarantine programme and establish the health-screening programme, which will be affected by the source of the animals. Details should be obtained of the groups in which they were kept and how long they had been established. If they were weaned at less than 12 months they will be more likely to be stressed and more likely to be carrying subclinical infections, which may then become a clinical problem when they arrive. Information should be obtained on the standards of care in the source establishment and the background health status of the source colony, taking note of the time period over which this been established. During the quarantine period incoming monkeys need to be isolated from existing stock. It is vital to set aside enough time to allow for observation of these new animals, building up a picture of each one's normal behavioural repertoire, so that changes due to illness or experimental stress may be spotted rapidly. The behaviour of juvenile long-tailed macaques does not settle to pre-transport levels even 1 month after international transport and relocation to a new unit[35].

Quarantine can have a considerable impact on animal welfare, particularly if there is single housing, small cage sizes and a lack of enrichment, isolation from former social companions, or fear of unfamiliar animals and humans in close proximity. If quarantine is required it is important to avoid unnecessary constraints on the animal's husbandry and care.

Health-screening programme

It is preferable to prevent diseases rather than to have to deal with them when they arise. A number of infectious diseases of primates are potentially zoonotic so health-screening programmes cannot only improve animal health and welfare but will also contribute to the occupational health programme. The health programme should also take into account any necessity to screen staff to prevent spread of infections from humans to monkeys.

Monkeys can carry a number of potentially serious infectious diseases: some bacterial, ranging from salmonellosis to tuberculosis, and some viral. The UK Advisory Committee on Dangerous Pathogens has made particular recommendations with respect to simian herpesvirus (Herpesvirus simiae, B-virus), and simian retroviruses[36]. A health-screening programme should be drawn up for every facility holding primates which should take account of the source of the animals, the use to which they are put (e.g. breeding/experimental, long term/short term, immunology/neuroscience). For the general principles of health monitoring see Chapter 5 in this volume and for more detail on primate health screening see Wolfensohn15 and Wolfensohn and Honess7. There are many diseases which should be included in a regular screening programme as discussed above. Full details on the diseases of primates and their management can also be found in Bennett et al.[37] and Wolfe-Coote[38].

Diseases

Infectious diseases

The potential of primates to carry serious zoonotic diseases should not be underestimated. Captive-bred animals of known health status are less of a risk, and these comprise the majority of animals used. Monkeys are susceptible to many diseases carried by humans, such as colds and flu, tuberculosis, measles and many others. Therefore it is important to exclude casual visitors or at least wear a suitable face mask to reduce the risk of spreading such infections to the monkeys if appropriate occupational health surveillance has not been established. Power hoses promote the formation of aerosols, which may allow transmission of zoonotic diseases, so suitable protective clothing must be worn when operating these.

Animals used in experiments should wherever possible be from herpes B-virus-free colonies, particularly where animals are handled under conditions of close contact, in long-term studies or for neurological surgery, or for work where a latent infection may be reactivated, such as those involving psychological stress or immunosuppression.

Enteric disease may be caused by a number of factors including pathogenic organisms. Monkeys will pass loose stools if they are frightened, or if there are changes in their environment or diet. Stress, such as after weaning, transportation, surgery or due to

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changes in social hierarchy, can also cause subclinical infections to become clinical, so any case of diarrhoea should be investigated to eliminate infectious causes. Zoonoses such as *Shigella*, *Salmonella*, *Campylobacter* and *Yersinia* are common bacterial pathogens. Husbandry and environmental factors may also contribute to the development of enteric disease, as will viruses such as rotavirus, retroviruses and haemorrhagic viruses, and parasites such as *Giardia* spp., *Trichomonas* spp., *Entamoeba histolytica*, *Cryptosporidium* spp. and *Balantidium* spp., and the metazoans *Strongyloides* spp. and *Trichuris* spp.[39]. Therefore, prompt and accurate diagnosis of the aetiology of the enteritis is important so that a specific therapy can be instituted.

Husbandry-related diseases

A management strategy for dealing with such problems as fight injuries or nutritional imbalances that may be encountered with increasing use of foraging and group housing should be developed and built into any primate health-management programme. The benefit of social housing is that the environment is dynamic, unpredictable and variable so there is little habituation, but there are increased risks of infection, wounding and competition for food. With good management strategies, such as appropriate housing, these risks can be minimised but not altogether removed.

Clinical problems may be brought about by the increasing provision of forage mix in addition to normal pelleted diet. Although the forage mixes are all well balanced nutritionally, this assumes that the monkey will not pick out its favourite bits and leave the rest, so the provision of forage has to be carefully managed to prevent deficiencies. By introducing an element of choice the diet may become unbalanced and problems, such as rickets in juvenile animals, may arise from this. Social rank may be associated with obesity[40] since the dominant animal determines feeding time and the subordinates eat afterwards, depending on spatial distribution of food and the mix of food types. Dietary restriction (but without essential nutrient deficiencies) will increase survival and delay the onset of degenerative ageing conditions.

Health and safety

Particular care must be taken when handling primates because of the danger of transmitting potentially zoonotic diseases. Appropriate protective clothing must be worn. For quarantine animals, this should include cap, gown, mask, boots and gloves. For other animals, gloves and protective gowns may be sufficient but there will be local safety rules for each institution which must be followed. These rules will take into account the origin of the animal and the results of health screening.

Laboratory Procedures

Handling and restraint

All primates can and will, on occasion, bite. They are very quick, surprisingly strong and will snatch and grab at such things as jewellery and loose clothing. Injuries can be prevented by knowledge of the particular species and the individual, including knowledge and interpretation of the posture and expression of the animal. The responses of different species of primates to environmental stressors varies and this is reflected in their response to minor procedures such as routine capture. When compared with rhesus, cynomolgus macaques tend to be more stressed[41], and the animals' responses to stress can have implications for research[42].

Restraint for any purpose and whether for short duration or longer may induce fear and stress responses, such as physical resistance to handling, alarm vocalisations, defensive threatening and aggression, urination and defecation, and there are also likely to be physiological responses that will increase unwanted data variability[25,43]. These fear and stress responses should be recognised as indicative of a serious welfare problem that must be properly addressed. It may be that restraint itself can be avoided. Habituation and socialisation, together with training animals to cooperate with procedures using positive reinforcement techniques, can obviate the need for physical or chemical restraint.

If restraint is required to control a primate during a scientific procedure then the method used should provide the least level of restraint for the minimum frequency and duration necessary consistent with achieving the experimental aims. It should protect both primate and personnel from harm and should avoid causing unnecessary distress or discomfort.

Restraint chairs are used to support primates in a sitting position when it is deemed necessary to restrain them for prolonged periods, such as for single-cell recording within the central nervous system or chronic infusion when they may be required to remain in one position for several hours. Chair restraint can affect the animal's physiology[44] and can severely compromise their

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welfare[45,46].

In order to carry out a full examination of a primate it may be necessary to sedate it in order to reduce the risk of injury to the handler, to reduce the stress to the animal and also to enable the examination to be carried out thoroughly to yield the maximum possible information. A 'restraint' cage may be used in which the back is pulled gently forward or the front pushed gently backwards. However, most animals find this procedure very stressful. They may be netted[47] but this is also significantly stressful and most animals will not respond well to this method of capture. It is much better to spend time training the monkey to present itself for the procedure, which can then be carried out without stress to animal or handler. Macaques can quite quickly and easily be trained to present their hindquarters for injection, or present a limb for blood sampling[43,48,49,50].

Even if a macaque is sedated it should be held by the upper arms, to keep its face away from the handler. Even better is to train the animal for manual capture and restraint since sedatives themselves are stressors13. Many experimental procedures require gaining access to the individual animal but primates can readily be trained to cooperate even when they are living in social groups. Training is encouraged by the use of positive reinforcement and they can also be trained using voice commands, which will not distort their nutrition.

Macaques can be trained to enter a transport container and other methods include catching animals in a nest box or transport container, or use of a pole and collar. These methods all have advantages and disadvantages and a useful discussion of the welfare implications of the various techniques is contained in Rennie and Buchanan-Smith[51]. Whatever the method of capture, macaques should be safely restrained and their body weight supported when they are carried.

Training

Training animals to cooperate with scientific, veterinary and husbandry procedures helps to reduce the stress that may be caused to both the animal and the laboratory staff. Primates are not domesticated animals and contact with humans can be extremely stressful, especially where the primates are not in control of the level and intensity of that contact, which is a particular problem in the laboratory14. Habituating and socialising captive primates to the presence (sight, sound, smell) and behaviour of humans as early as possible in their lives is essential. Otherwise, both animal welfare and the science can be seriously affected. Early habituation reduces any fear or distress the animals may experience when confronted with new situations as adults. It also allows staff to observe behaviour patterns which are relatively unaffected by their own presence, and this helps them to assess the welfare of the animals more effectively.

There should be a formal habituation and socialisation programme, based on the principles of positive reinforcement[52]. In addition, all staff that come into contact with the animals should understand the need to ensure that their own actions make a positive and consistent contribution to the habituation and socialisation programme, and everyone should be trained accordingly. However, in order to start a programme of training with laboratory primates it is first necessary to train the people to communicate and interact positively with primates through recognition and interpretation of primate signals, and to help the primates to respond positively to humans through habituation and socialisation.

Training methods should be based on positive reinforcement techniques, which reward desired behaviour, since this method of training is considered to be the most humane[53,54]. Negative reinforcement should only be used when positive alternatives have been exhausted (e.g. training for aversive procedures), and it should only be used in combination with positive reinforcement.

Positive reinforcement training refers to any form of training that is based on rewards rather than punishment. The animal is rewarded with some treat, such as a morsel of food, for a job well done. Negative reinforcement is the opposite of positive reinforcement and involves a punishment for less than favourable performances and usually implies some kind of fear, pain or discomfort for the animal being trained. Another way to get an animal to perform a task is to motivate it by withholding food or water and it has to perform the task in order to obtain food or water. As the intake of water is essential for survival, water deprivation is a stronger motivator than food deprivation and will achieve faster results since an animal will become physiologically compromised quicker with water deprivation than with food deprivation. Access to fluid in these conditions cannot be described as 'positive reward'.

Fluid regulation may simply involve strict scheduling of the access to water, or may involve restriction in which the total amount of water is strictly controlled, so that thirst becomes the motivator for performance of certain tasks. With limited access to water, food consumption also decreases and these protocols are often associated with weight loss and associated stress. On such protocols the amount of fluid consumed and a hydration assessment should be recorded daily for each animal. Variables that can be used to assess hydration status include the body weight, food intake, skin turgor, urine output, moistness of faeces, general appearance and demeanour. It is important to evaluate the health and welfare of each individually[55]. Animals should generally be given free access to water for some period of time on days when there are no experimental sessions33. There is currently much

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debate over the use of water restriction paradigms[56–59].

Administration of substances, blood sampling

Some recent reviews have been published which provide detailed advice on refinements for the administration of substances and of sampling[60–63]. Factors to consider include whether the particular individual or species is the most appropriate for the study or whether it is too easily stressed by handling and requires further time to acclimatise to the procedures. Pre-screening should be conducted to determine whether the particular animal's temperament is conducive to training and to performing the tasks required. The use of sedatives may or may not reduce stress13, but may confound the experiment and not be feasible. If possible animals should be trained to cooperate with the procedures to avoid the need for physical restraint. Staff should be competent to carry out the procedure and post-administration monitoring.

Telemetry

Telemetry is frequently used in studies involving primates, including those carried out to fulfil the requirements of regulatory bodies. Telemetry is widely viewed as benefiting science and animal welfare because it can reduce stress caused to animals (e.g. by restraint), enable reductions in animal numbers and provide indicators of animal well-being to help implement humane end points. However, telemetry can require invasive procedures such as implantation surgery, single housing and use of jackets, which can cause pain and distress. Although telemetry is often described as a refinement, this will not be the case unless the technique itself has been fully refined. The use of telemetry may also increase the time for which an individual animal is used on procedure and the cumulative severity must therefore be taken into account[64].

Detailed advice on refinements in telemetry can be found in Morton et al.[65] and Hawkins et al.[66] including maintaining stable group housing, and behavioural pre-screening to ensure that individuals are suitable for projects and are not implanted unnecessarily.

Anaesthesia, analgesia and post-operative care

Before anaesthesia primates should have food withheld for at least 6 h, and for Old World monkeys it is important to check that the cheek pouches are empty to ensure that the animal does not choke on recovery. A selection of drugs that may be used to sedate primates to facilitate handling are listed in Table 9.3g. Veterinary advice should be sought on the use of these but some further information is provided by Foster et al.[67] and Sun et al.[68]. In the UK, all these drugs are prescription-only medicines and therefore must only be used under veterinary direction.

Induction (and maintenance) of anaesthesia can be by intravenous infusion of propofol. Barbiturates are associated with respiratory depression and resultant hypercapnia. Primates should be intubated, having first sprayed the larynx with local anaesthetic to prevent larygospasm. Prior to intubation, administration of oxygen by face mask is advisable. Maintenance of anaesthesia using isoflurane or sevoflurane is most satisfactory and gives a good quality of recovery. During anaesthesia it is essential to monitor the vital signs including temperature, blood pressure, oxygen and carbon dioxide levels and maintain physiological stability with administration of fluids. Post-operatively, analgesia can be provided with opioids or non-steroidal anti-inflammatory drugs. Post-operative gastritis and vomiting can be controlled using metoclopramide. Selection of the appropriate regime will also depend on the area of scientific investigation as some anaesthetics may affect the area under study[69].

Husbandry post-surgery

Animals may need to be allowed to recover separately in the immediate post-operative period. However, it has been successfully demonstrated that surgically treated animals can be pair or group housed soon after recovery[70] and they should be returned to their social group as soon as possible.

Interrupted subcuticular sutures, with the possible additional use of tissue adhesives, should be sufficient to maintain the integrity of a surgical wound such that removal of sutures by the individual or cage mates is not a problem. Adequate perioperative analgesia will prevent animals from paying untoward attention to their own wound sites and attracting the attention of others. It has also been shown that primates with surgical implants, for example cranial implants, can successfully be kept in social groups70. For work on extremities and limbs primates will tolerate bandaging to provide protection and support of the affected area provided the dressing is carefully applied.

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When planning housing for the recovery period, remember that primates may climb before they are fully recovered from anaesthesia, and may fall and injure themselves. A purpose-built recovery enclosure may be used or a temporary reduction in the height of the home area may be implemented. If a recovery enclosure is used, it should be constructed so that the primate does not have to be physically caught in order to be returned to the home cage, or animals should be trained to enter and leave a transport cage. Animals should be allowed to recover in proximity to others from social group, not in social isolation. If surgery involves producing neurological lesions, the home area may need to be adapted to take account of any possible long-term side effects. If single housing following surgery can be justified, individual animals should have visual, auditory and if possible tactile contact with other primates.

Welfare

There are a number of parameters that can be used to assess welfare, which may be behavioural, physiological or biochemical (for more information see Chapter 4). The most readily accessible and commonly used index for assessing well-being in primates is behaviour.

Natural behaviour

All the primate species commonly used in research are highly social animals. Old World primates are typically female kin-bonded groups with the distribution of males superimposed onto that of the females. In the wild the group structure varies according to differences in the quality and density of resources and incidence of disease and level of predation. Knowledge of the normal social structure (including how groups are constituted and dominance hierarchies established) should be used to inform how they should be group housed. This is important in determining group size and structure, defining socialisation programmes, managing aggression, promoting natural behaviour and optimising reproductive success[71].

Primates live in social hierarchies in which there are inevitable conflicts and fights will occur in which some animals may be injured or occasionally even killed. A singly housed animal may be in excellent physical health, but single housing is a major stressor and the animal will suffer the stress of social deprivation, and such animals frequently show a poor behavioural repertoire with abnormal and even self-harming behaviours and do not have well-developed coping skills. In nature, many primates live in fairly large groups (around 50 in a troop for macaques) and while putting a small group of animals or a pair together will give them company, there appears to be a higher incidence of fights and unsettled behaviour in these than in larger groups, even if floor space per monkey is lower in the latter case. A pair is not a normal social group.

The components of a wild macaque's diet typically come in small aliquots (e.g. seeds, buds and flowers), dispersed over a wide area, so the process of gathering them is very time consuming and demands much energy. The daily activity cycle reflects this as a macaque troop typically spends the morning foraging, followed by a rest period, then another bout of food gathering through the afternoon, finally moving to a sleeping site for grooming and sleep. Of the two macaque species commonly used in research, the cynomolgus monkey spends more of its time in the trees whereas the rhesus is a ground-dwelling monkey that forages on the floor and takes to the trees for safety and to sleep. Macaques also appear to enjoy playing in water and, although they do not actively swim, they will forage for food in shallow water and use water for thermoregulation.

Primates communicate in a variety of ways using sounds, facial expressions and postural changes. Many signals can be easily interpreted. For example, shaking a tree is a sign of aggression which, in a captive environment, translates to rattling the cage. A low-grade threat can be communicated in some primates with a brief ‘eyebrow flash’ which in humans is an indication of recognition and may form part of ritualised flirting behaviour. Raised eyebrows combined with lip-smacking are used as a social appeasement signal in macaques. Other facial expressions may communicate quite different meanings to non-human primates than to humans. For example, the grimace, or silent bared-teeth face, indicates uncertainty or submission in many primates but, as a smile in humans, can be seen as an expression of friendliness. Another common source of confusion is the human ‘stare’. A person may stare at an animal out of interest, but most primates will interpret this as an aggressive threat and respond accordingly. This ‘miscommunication’ can be a problem when people who have no experience of interacting with primates visit animal units.

Assessment of welfare

Primates are highly intelligent, sentient and social animals with a complex range of physical as well as psychological needs; therefore both physical and psychological well-being should be considered when assessing welfare. There is no doubt that they

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experience pain and a range of negative psychological states such as anxiety, apprehension, fear, frustration, boredom and depression[72], as well as a range of positive states. Mood can also be affected by social status[72]. It should be assumed that procedures that cause pain and distress in humans will probably cause pain or distress in other primates[73,74]. A monkey in pain may show a generally miserable appearance, and may adopt a huddled position or crouch with head forwards and arms across the body. It will tend to refuse food and drink and to avoid companions, although monkeys that are unwell may attract extra attention from cage mates, varying from social grooming to attack. Vocalization is more likely to indicate anger than pain.

The health should be evaluated regularly and records kept and a programme to review and improve physical and psychological health applied where necessary. Evaluation of the psychological condition of the animal should take into account its physical health, its behavioural repertoire and an assessment of its coping skills when presented with novelty. The latter will evaluate normal inquisitive and explorative behaviour or any abnormal response and how long the animal takes to return to baseline level of behavioural repertoire. All staff–animal interactions should be based on an understanding of species-typical behaviour patterns and communication systems, such that these are interpreted correctly and responded to appropriately.

Clinical scoring systems are usually used for monitoring of single incidents but there should also be some assessment of the cumulative suffering and the lifetime experience of the animal. This will include direct suffering from the procedure as well as contingent suffering[75] as a result of transport, housing, the environment and injuries from caging or conspecifics, to name just a few examples. The life span of primates, compared with other laboratory animal species, is relatively long. They are valuable animals, have long breeding lives and are likely to be used in long-term experiments. Some primates may therefore be housed in the laboratory for many years. In such circumstances there are particular welfare issues that must be addressed65,66. A welfare assessment matrix can be used to give an estimation of cumulative suffering and a pictorial view of welfare[76].

Physical health

The first step in assessment of the monkey's physical health is to observe the animal in its home cage and evaluate its appearance, behaviour and general demeanour. In order to do this it is vital that the observer has some experience of the animal (both the species and the individual) to be able to judge whether the animal is exhibiting a normal behavioural repertoire. Just like humans some individual non-human primates exhibit behavioural patterns that are specific for that individual, but do not necessarily reflect poor well-being. Only after completing the examination from a distance and noting the behavioural responses should the animal be caught to enable it to be examined more closely. Catching it will markedly affect its behaviour, whether or not sedatives are used, which it why it is important to make a full evaluation first. For a full description of clinical assessment of a monkey see Wolfensohn and Honess7 and Smith et al.55.

The animal's weight should be recorded and its body condition scored. It is assessed by palpating the monkey over its thoracic and lumbar vertebrae (at the level of the last rib) and making a judgement as to the amount of fat and muscle covering the bony prominences of the vertebrae and giving a quantitative score. Condition scoring at weaning and during the post-weaning period, combined with regular weight measurements, is important to ensure that the animal is receiving adequate nutrition and growing properly.

Psychological health

This may be assessed by behavioural monitoring and by monitoring conditions such as alopecia[77]. When assessing welfare using behavioural parameters it is important to assess the animals at various times. The animal's behaviour should be noted on the approach of the observer. Observations should then be repeated after the animal has had time to habituate to the presence of the observer. The use of space and structures, any self-maintenance behaviour, and social behaviour, as well as the animal's disposition and interaction with observers, are all recorded.

An awareness of the normal behavioural repertoire for the species is important to enable provision of appropriate care and management and for assessing their physical and psychological well-being. Knowledge of the behaviour of individual animals is also essential since primates show a high degree of individuality. Any deviations from normal must also be recognised to enable evaluation of refinements such as changes in cage design or environmental enrichment.

The advice of a primate behaviour specialist is invaluable in training both care staff and research staff to recognise and interpret deviations from normal behaviour and to assist in developing programmes to ensure good psychological health.

Euthanasia

The principles of euthanasia are set out in Chapter 8 in this volume and in Close et al.[78,79], American Veterinary Medical

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Association 80 and Wolfensohn[32], all of which refer to the animal welfare concerns with respect to carrying out of euthanasia. Having to kill a primate may be a more emotionally challenging experience for staff than having to kill some other species. Staff who carry out this procedure must not only be competent in the appropriate methods, but it is essential that they receive good training, adequate supervision and any necessary empathetic support.

The most common method for killing primates is an overdose of an anaesthetic, such as sodium pentobarbitone (at 100 mg/kg) administered intravenously. For larger primates it will be necessary to sedate the individual before attempting an intravenous injection.

When primates are euthanased, every effort should be made to make full use of their tissues and blood, particularly if this will minimise the number of animals having to be used. This requires good mechanisms for communication to match supply and demand both within and between establishments. Establishing tissue banks and data-exchange networks is one means of coordinating, optimising, reducing and refining primate use. In the USA www.primate.wisc.edu/pin/achieves this objective and in Europe EUPRIM-NET offers a tissue and gene bank (www.euprim-net.eu/).

Record keeping

All primates should be uniquely identifiable, for example by microchip or tattoo. A file or 'passport' giving full details on each individual primate should be maintained. It should keep the details of their biography enabling a full picture of previous experiences to be established. This should be sent with the animal if it is moved between institutions, together with general information on the establishment of origin, such as details of animal care and routine procedures, in order to assist in continuity of care and to ensure successful acclimatisation to the new facility. Good record keeping is essential for retrospective review and for the assessment of cumulative severity.

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Chapter 14

Production (Farm) Animals

C.J. Trower

Production animals are those that are farmed to produce food for human consumption (meat, milk, eggs), or animal by-products (wool, fibre, hides). They include a number of domestic ungulates (sheep, goats, pigs and cattle), as well as several species of poultry and some farmed fish.

Farm animals have several disadvantages as research tools when compared with the more common laboratory animal species. They are generally outbred populations, with many different breeds and cross-breeds and the majority of them have a poorly defined health status. All of the ungulate species have gestation periods measured in months, rather than days, and, with the exception of pigs, they also have very small litter sizes. These factors can make it extremely difficult to source large numbers of healthy farm animals that are matched for breed, sex and age.

In 2010, farm animals accounted for 5% of the 3.7 million procedures started that year in the UK[1]. The majority of these were poultry species (133 000), with smaller numbers of sheep (38 000), pigs (2700), cattle (2100) and other ungulates (100).

Poultry

Poultry is a generic term used to describe birds that are kept for meat or egg production. The predominant species of poultry is the domestic chicken, but the term also includes turkeys, ducks, geese, bantams, partridges, quail, pheasants, guinea fowl, ostriches, emus, rheas and pigeons.

In 2010, domestic chickens accounted for 91.5% of all the procedures carried out using avian species in the UK1. Many viruses are propagated in embryonated chicken eggs; for example, influenza viruses. Turkeys were used in a further 1.8%, whereas quail, ducks and geese (both domestic and wild), pigeons and the red jungle fowl accounted for only small numbers. Other non-poultry birds (finches, tits, starlings, crows, wild seabirds and the pied flycatcher) made up the remaining 6%. A simple classification of the majority of birds used in research is given in Figure 14.1.

Figure 14.1 Classification of birds.

Class	Order	Species
Aves	Galliformes	Domestic chicken (*Gallus gallus domesticus*)
		Domestic turkey (*Meleagris gallopavo*)
		Common quail (*Coturnix coturnix*)
	Anseriformes	Ducks and geese
	Struthioniformes	Ostriches
	Columbiformes	Pigeons

Poultry may be purchased from any commercial supplier. Poultry breeding companies in the UK will supply fertilised eggs or day-old chicks. Poultry flocks may have high health status or be of conventional status with unknown pathogen load. Researchers should always establish the health and vaccinal status of the supply flock.

By law, all poultry premises with more than 50 birds are required to register with Department for Environment, Food and Rural Affairs (DEFRA). Registration can be carried out on-line at http://poultry.defra.gov.uk.

Biology

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External features

There is a keratinised beak, without lips or teeth. The legs are covered in scales, and the remainder of the skin is covered in feathers, of which there are five types, each modified for a different purpose, such as flight, insulation, camouflage and sexual attraction. There are no sweat glands, but an oil gland (preen gland) is located on the back at the base of the tail. Female chickens also have a brood patch; an area of featherless skin on the underside of the abdomen, which is well supplied with blood vessels to transfer heat to the incubating eggs.

Skeleton

The skeleton of birds must be light enough to fly and yet strong enough to support the large pectoral muscles required for flight. To achieve lightness, the medullary cavities of some of the long bones are filled with extensions of air sacs. However, this leaves the cortices of the bones considerably thinner than those of mammals, which make the bones quite brittle and susceptible to fracture. The forelimbs are modified as wings, with fusion of many of the bones to provide additional strength. The sternum of birds is massive, to allow for the attachment of the muscles of flight.

Cardiovascular system

The heart is four-chambered, and larger in proportion to body weight than that of mammals. All avian red blood cells possess a nucleus.

Respiratory system

Birds do not possess a diaphragm, and the lungs are non-lobar and do not expand as in mammals. In chickens, the lungs connect with nine air sacs, some of which extend into the long bones. Birds breathe by expansion and contraction of the chest wall, acting as bellows. Air passes through the lungs into the air sacs during inspiration, then through the lungs again on expiration. Gas exchange in birds is highly efficient, taking place on both inspiration and expiration.

Digestive system

In birds there is an oropharynx, with no soft palate. In many birds, including poultry, the oesophagus has a muscular dilatation half way along its length called the crop. This acts as a food store, and softens and regulates its flow. The stomach is in two parts, the secretory proventriculus, and the muscular ventriculus or gizzard, which grinds the food. In chickens kept outdoors the gizzard will contain grit to assist in this process. There is a short colon with two large caecae.

Renal system

The products of renal excretion are thick and pasty, high in uric acid, but with low water content. The chicken does not have a bladder, so this urate paste is excreted via the ureters, which open directly into the cloaca. The urate paste shows as a white cap on some faecal stools. Kidney failure will result in more dilute urate paste, which can be mistaken for diarrhoea.

Reproductive system

Female birds have only one functioning ovary and oviduct (the left), leading directly to the cloaca. The male has two intra-abdominal testes located by the kidneys. The ducta deferentia open into two papillae on the wall of the cloaca.

Domestic Chicken

The domestic chicken, *Gallus gallus domesticus,* is a subspecies of the red jungle fowl. It is by far the most numerous species of bird on Earth, with an estimated 50 billion worldwide. In the UK approximately 800 million chickens are killed and 100 000 tonnes of eggs are processed every year for human consumption.

Chickens possess a fleshy protuberance on the top of the head (the comb), and two fleshy protuberances under the chin (the

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wattles). These can vary in size and shape between breeds, but are usually larger on the male than the female. They act as thermal regulators, and the comb is also a sexual display organ in males.

Behaviour

Chickens are ground-living birds, unable to fly for more than short distances. They are highly social and form stable groups. The behaviours that are most important to them are nesting, perching and using substrate for scratching, pecking and dust-bathing. Given the opportunity, chickens spend a great deal of time performing foraging behaviour (pecking and scratching the ground), and foraging opportunities should be provided for chickens housed in the laboratory. Feather pecking, which can be seen in cage kept birds, is believed to be misdirected foraging behaviour, rather than aggression.

Housing

Chickens should always be kept on solid floors with a suitable substrate if possible. If cages with grid mesh floors must be used, then birds should be provided with a solid resting area occupying at least a third of the cage floor. Perches should always be available and nest boxes provided for laying birds over 16 weeks of age. Note that some hybrid strains of broiler chicken can grow very quickly, so good forward planning is required to ensure that adequate space is available for the duration of the experimental period.

Cleaning regimes will vary according to the type of accommodation. If kept in deep litter systems this is usually wood shavings or straw, which should be dry and friable. If drinker management is poor, or the ventilation is inadequate, the litter can become wet or consolidated. Bird droppings will then remain on top of the litter, which will in turn lead to soiling of the birds, scabby hocks and breast blisters, and will also predispose to respiratory infections.

Dust and dander from domestic poultry may present a risk to people with allergies or respiratory infections. Appropriate cleaning regimes, ventilation and protective clothing are therefore required.

Feeding

Chickens bred for prolific egg laying tend to be small, whereas those used as broilers or broiler breeders (meat-type) have rapid growth rates and a large body size and are less efficient egg layers. Nutritional requirements differ for these two kinds of chickens. Note that some commercial feeds contain a coccidiostat to prevent coccidiosis, and some coccidiostats for chickens can be toxic to turkeys.

For growth, laying chickens need a diet containing 18% protein initially, reducing to 16–17% as egg laying approaches (approximately 20 weeks). Diets are usually based on cereals, with a metabolisable energy content of approximately 2850 kcal (11 930 kJ)/kg of diet

Broiler chickens need up to 23% protein initially, reducing to 18% by weeks 6–8. The diet should have a metabolisable energy content of approximately 3200 kcal (13 400 kJ)/kg of diet[2].

Poultry are sensitive to abrupt diet changes, so changes in feed must be made gradually by mixing the feed in decreasing proportions of ‘old’ feed over a few days. In some birds the feed intake may be restricted to control growth rate, as too rapid growth in broilers can cause lameness, or circulatory problems. It is important to feed the correct rations, and with restricted diets particularly there must be adequate space for all the birds to feed at the same time.

Newly hatched chicks need to be encouraged to feed by placing feed on chick paper, or on flat trays, where they can walk and feed easily. Chicks can survive for the first few days on the remnants of the yolk sac, but if they are not feeding by day 4 they will die of starvation (known colloquially as ‘starve out’).

Feeders must be cleaned out regularly, or food will go mouldy, risking the production of mycotoxins.

Water

This is usually provided by automatic drinkers. One nipple or cup drinker should be provided for every four birds, with a minimum of two in every enclosure. They must be cleaned regularly to prevent the build-up of pathogens. As the birds gain weight the drinkers must be raised such that the water is available at head height. Leaking and overflowing drinkers must be avoided.

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Environment

The light intensity and the total length of photoperiod controls egg laying. Under natural lighting chickens often lay throughout the winter in their first season, but will go out of lay in the autumn in subsequent years, starting again as the days get longer. Increasing light intensity and photoperiod can prolong the egg-laying period. Feather pecking can be a problem in group-housed chickens, and subdued lighting has been used as a control measure to reduce the incidence of this. However, this should be discouraged, and a lighting level of at least 20 lux is recommended[3]. Feather pecking is better controlled by providing adequate environmental enrichment. Any increased activity resulting from the use of higher-intensity lighting can be offset by using red filters, which the birds see as dark. Wherever possible there should also be a dawn and dusk period.

Chickens should be kept at temperatures between 15 and 24°C, depending on age3. Additional heat lamps may need to be provided for young birds, in which case the birds must always have sufficient space to regulate their own environmental temperature by moving away from the lamp.

It is very important to provide efficient ventilation without draughts. Inadequate ventilation results in a build-up of airborne dust, microorganisms, water vapour and ammonia. The specialised respiratory system of birds makes them particularly vulnerable to inhalation of particulate matter and the development of respiratory infections. The presence of a significant odour on entering poultry rooms is a good indication that the air-handling system is not functioning correctly.

Birds are more tolerant than mammals to changes in relative humidity and a range of 40–80% is generally acceptable. However, extreme variation in humidity can affect the rate of heat loss in birds, which will influence feed intake and activity.

Birds are susceptible to stress and sudden or prolonged noise can have a detrimental effect on their welfare and production. The general background level of noise in a room should be less than 50 dB, below a noise-rating curve of 45, and free from distinct tonal content.

Breeding

Female chickens begin egg laying from about 18 weeks of age, and males begin to produce semen from as early as 8 weeks. Variation of the photoperiod and temperature are used to stimulate egg production in both laying and breeding birds. Most chickens bred for commercial egg production will lay an egg nearly every day from about 18 weeks of age, depending on satisfactory growth and light levels.

Under natural conditions hens lay until a certain clutch size is reached (usually 12), and then stop laying to incubate all the eggs. She will sit on the nest, and defend it if disturbed, and may not leave the nest to eat, drink or dust-bathe. The hen maintains the nest at a constant temperature and humidity, and turns the eggs regularly.

After mating, semen can take several days to reach the infundibulum, where fertilisation takes place. The fertilised oocyte enters the oviduct, where the layers of albumen are added first, followed by the two shell membranes. The main part of the shell, composed principally of calcium carbonate, is added in the shell gland at the end of the oviduct, over a period of about 20 h. The whole process from fertilisation to laying the egg takes 24 h.

The chicken embryo then requires 21 days incubation before it is sufficiently developed to hatch (28 days in turkeys). However, fertilised eggs will not begin development until the temperature rises above 21°C, and if they are stored at 12–15°C and 75% humidity they may be kept fertile for up to 14 days. The temperature can then be raised to 37.5°C by putting them in an incubator, which is known as ‘setting’ the eggs. Best results are obtained if eggs are incubated within 4–7 days of laying, and fertility drops sharply if they are stored beyond 14 days. During incubation the eggs must be kept warm and occasionally rotated. Incubators are closely controlled for temperature, humidity and ventilation, and the egg trays also gently move periodically, to mimic the action of the hen. Temperature regulation is critical, and variation from the optimum of 37.5°C reduces hatch rates. Relative humidity is also important, because this controls evaporation. Evaporation causes contraction of the fluids in the egg, resulting in the formation of an air sac between the two shell membranes. The size of the air sac, and thus the degree of evaporation, can be assessed by candling. This involves shining a bright light through the egg shell, and can be used to assess the viability of the developing embryo and the network of blood vessels surrounding it.

For best results eggs should be placed with the pointed end down and turned regularly (at least three times per day) until 1–3 days before hatching. If the eggs are not turned the embryo may stick to the shell, resulting in physical defects in the chick. Adequate ventilation provides the embryo with oxygen and prevents build-up of carbon dioxide.

Handling

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Domestic chickens are normally docile and do not usually present a threat to handlers. However, they can peck, scratch or hit out with their wings, so caution is required. They are more used to human contact than most species of bird, but can become distressed if they are not handled competently. Rough handling may result in bruising or broken bones, whereas holding birds too tightly may restrict the movement of the chest wall, preventing respiration and causing asphyxia.

Chicks and young birds up to 7 weeks of age should be approached quietly and a hand placed over the bird's back to gently restrain the wings against the body. Once held in this fashion they can be turned over, so that the chick is restrained on its back.

Adult chickens are most easily caught by placing a hand on the back and restraining the wings. Lift the bird by supporting under the body and restrain the bird by holding it against the chest. Once restrained, the hands can then be transferred to take hold of both of the legs, to support the bird's weight (Figure 14.2).

FIGURE 14.1 Handling a chicken. (a) Pick the chicken up with both hands, ensuring the wings are restrained. (b) Hold the chicken against the body, restraining the wings and legs.



Warning: do not catch any bird by the legs, as this will lead to flapping of the wings with possible damage to the bird or the handler.

Techniques

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Identification

Poultry are best identified by dyeing the feathers, or by the use of open or closed leg rings. Wing tags and electronic tags can be used, although they are more invasive. When leg or wing tags are used on chicks care must be taken to allow for growth.

Administration of substances

Intravenous

Intravenous injections are often carried out via the alar (wing) vein, found on the underside of the wing, running across the surface of the humeral–radio–ulnar joint, directly beneath the skin. Alternatively, the jugular veins run either side of the neck. Featherless tracks of skin overlie the veins and can help identification of their position.

The medial metatarsal vein is situated on the medial side of the lower leg. Care should be used with this site, as it may cause lameness.

Intramuscular

This should be performed into the pectoral muscles, which are situated on either side of the sternum. Birds have a renal portal circulation, which means that substances injected into the hind quarters of a chicken may be transported by the femoral vein to the kidneys and excreted, before entering the general circulation. For this reason it not advisable to use the leg muscles for injections.

Subcutaneous

Subcutaneous injections should be given under the skin over the keel bone.

Oral

Oral dosing is performed using a needle with a pear-drop end.

Inoculation of chicken eggs

The injection site must first be determined by candling and a small hole drilled through the shell using a glass cutter. A fine needle (13 mm, 27 G) is then passed through this hole to the required site of administration. A needle guard is used to prevent too great a penetration. The hole in the shell is sealed with melted paraffin, and the eggs are returned to the incubator.

Collection of blood

Blood is normally collected from the alar vein, or from the jugular vein if larger quantities are required. Blood removal must be carried out carefully to minimise haematoma formation. The blood may be collected directly into a micro-hematocrit tube.

Cardiac puncture must only be used for removal of blood as a terminal procedure under general anaesthesia and be followed by an approved method of euthanasia. Place the bird on its back and insert a 20–22 G needle from the neck along the ventral floor of the thoracic inlet, avoiding the crop.

Pain and stress recognition

Responses to pain in birds are more subtle than with most other species. They often adopt a hunched posture, and display wing droop. The feathers become ‘ruffled’, which may be exacerbated by lack of grooming, and the bird will separate itself from the cage mates. They may develop an increased respiratory rate and ‘mouth breathe’ when distressed. There may also be changes to the colour of the wattles or comb and evidence of oculo-nasal discharges, and the droppings may become looser.

With severe pain, birds can develop a catatonic response, where they become immobile and totally unresponsive. With chronic pain there will be a loss of appetite and weight, which can best be measured by weighing the bird on a daily basis.

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Common diseases and health monitoring

Zoonoses

Domestic poultry suffer from a number of zoonotic diseases. *Campylobacter* and *Salmonella* do not usually cause clinical disease in poultry, but may cause disease in humans. Ornithosis (psittacosis), caused by *Chlamydophilia psittaci*, is a serious, potentially fatal, respiratory disease of humans that can be carried by many types of birds, including domestic poultry. Avian influenza is primarily a disease of birds, but there have been a number of human cases, mostly in south-east Asia, in people who have been closely associated with infected birds.

Clinical diseases of birds

There are many bacterial, viral, parasitic and metabolic diseases of domestic chickens.

Newcastle disease (fowl pest) and avian influenza can both cause rapid, unexplained, high mortality in flocks. These are notifiable and suspicion of these diseases must be reported immediately to DEFRA.

Many of the most serious chicken diseases are caused by respiratory pathogens. These include mycoplasmosis, infectious bronchitis (coronavirus) and infectious laryngotracheitis (herpesvirus). There are vaccines available for these diseases. Mareks disease and avian leucosis are also important viral infections in chickens.

Coccidiosis is extremely common in chickens. Infection with any one of nine species of this protozoan can result in weight loss, diarrhoea and death. Other parasites that infect poultry include nematode worms, such as intestinal, caecal, gizzard and tracheal worms, and ectoparasites, such as red mites and scaly leg mites.

There are a number of vaccines available for use in chickens and turkeys and these are widely used in commercial poultry farms. It is wise to check with the supplier to ascertain which diseases purchased chicks may have been vaccinated against.

More information on poultry diseases can be found at www.thepoultrysite.com.

Biological data and useful reference data

General husbandry data for chickens are given in Table 14.1. Values for feed intake and water consumption will vary with the type of bird kept and the accommodation of the birds.

Table 14.1 Biological data: chickens.

Biological data	
Adult weight (kg)	1.5–4
Diploid number*	78
Food intake (g/day)	125–250
Water intake (ml/day)	200–300 (should provide *ad libitum*)
Natural lifespan (years)	5–8 (can live up to 30 years)
Rectal temperature (°C)	41.5 (41–43)
Heart rate/min	180–250
Blood pressure, systole (mmHg)	71–95
Blood volume (ml/kg)	60–90
Respiratory rate/min	15–25
Breeding data	
Sexual maturity (weeks)	18–24 female, from 8 weeks in male
Age to breed (weeks)	20–23
Incubation (days)	20–22
Clutch size	1–14, if eggs removed birds lay daily for up to 1 year
Weight at hatching (g)	50–70 g
Haematological data	
Red blood cells ($\times 10^6/mm^3$)	2.5–3.5

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PCV (%)	22–35
Hb (g/dl)	7–13
White blood cells ($\times 10^3/mm^3$)	12–30

Hb, haemoglobin; PCV, packed cell volume.
*Birds have a number of macrochromosomes that are of normal size, and also microchromosomes which are of reduced size and are difficult to count. This figure includes 60 microchromosomes.

Anaesthesia of chickens

All avian species present special difficulties with regard to general anaesthesia. Birds have higher metabolic rates and higher resting temperatures than mammals of equivalent size. The higher temperatures lead to greater heat loss during anaesthesia. The high ratio of surface area to body weight also increases the heat loss, so it is particularly important to minimise this during anaesthesia by maintaining a high environmental temperature and providing insulation. Avoid removing large numbers of feathers and chilling the body by application of fluids, such as surgical spirit.

Fasting pre-anaesthesia should generally be avoided as the higher metabolic rate of birds can induce hypoglycaemia. This is particularly important for birds under 1 kg body weight.

Induction of anaesthesia may be by injection or inhalation. Chicks may be induced by placing them in an induction chamber. Once anaesthetised they may then be maintained via a face mask or an endotracheal tube.

The two-stage respiratory cycle of birds and the presence of air sacs can allow a build-up of anaesthetic gases in the dependent areas of the respiratory system, which may lead to anaesthetic overload. Care must be taken not to obstruct respiration, as even short periods of apnoea can be fatal. Avoid taping the wings and legs in full extension, as this can inhibit both respiratory movements and venous return.

Birds should recover in a quiet dark environment at a temperature maintained at 40°C. All cage furniture must be removed to avoid injury. It is also a good idea to temporarily fix the wings by taping them to the body with micropore tape, to prevent damage from flapping during the recovery period.

Warm isotonic saline may be given by subcutaneous or intravenous administration, at 5 ml/kg. Birds should be encouraged to eat as rapidly as possible after recovery. Unfortunately there is little known about the effectiveness of analgesia in birds. Dose rates for anaesthesia in chickens are given in Table 9.3d.

Ruminants

Ruminants are kept worldwide for their meat, milk, skins and wool (sheep), or hair (goats). The ruminant most commonly used for scientific studies in the UK is the sheep, but small numbers of cattle, goats and deer are also used. Figure 14.3 shows a simple classification for ruminant species. Note that species in the family Camelidae, in the suborder Tylopoda, are not true ruminants. This includes camels and the South American llamas, alpacas, guanacos and vicunas.

Figure 14.3a Classification of ruminants.

Order	Suborder	Family	Species
Artiodactyla	Ruminantia	Bovidae	Sheep (*Ovis aries*) Goats (*Capra hircus*) Cattle (*Bos taurus*)
		Cervidae	Deer

Ruminants are generally acquired from commercial suppliers, which are normally commercial farms. It is not recommended that ruminants are purchased through markets or dealers, as these animals will usually have been derived from a number of different farms, with no medical or husbandry history.

Most commercially farmed ruminants are reared under conventional conditions and the husbandry and health of such animals can be extremely variable. Whenever practicable, veterinary inspection and health screening of the animals should be undertaken on the farm of origin before transport to the research facility. It is advisable to isolate all animals on arrival for a period of 4 weeks, during which time clinical assessment, tests and treatments may be carried out. The following general rules should be

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observed whenever possible:

- purchase from as few sources as possible,
- purchase as infrequently as possible,
- ensure the source herd is free of pathogens that are significant for the species and the research,
- isolate incoming stock for as long as possible (4 weeks, if possible),
- during isolation, medicate for endemic pathogens, such as ecto- and endoparasites.

To maintain the health status of an established farm-animal facility it is essential to impose a continuing high level of biosecurity. Personnel and animals, and feed, bedding and other equipment, are potential sources of disease. The following procedures are advised.

- Visitors should be kept to the absolute minimum and should not enter within 48 h of same-species contact. As a minimum, a full change of protective clothing should be provided before entering the unit.
- Loading and unloading procedures must be strict. All livestock vehicles present a potential disease risk.
- The proximity of other animal units and major roads should be considered when siting a new unit. Some airborne pathogens can be carried for several kilometres.

Sheep

The sheep (*Ovis aries*) was derived from the mouflon (*Ovis orientalis*) by domestication over 10 000 years ago. There are more than 200 sheep breeds worldwide, with over 60 of these found in the UK. They vary in size from small native breeds such as the Soay or Shetland (adult female 25–30 kg), to the much larger down breeds, such as the Suffolk and Oxford (adult female 85–90 kg). Breeds also differ widely in temperament and fleece characteristics, and may be either horned or naturally polled. There are approximately 14 million sheep in the UK, the majority of which are bred for meat (lamb) production. Most of the breeding females are actually F_1 hybrids of two pure breeds. These ewes are then crossed with a terminal sire to produce an F_2 lamb for slaughter. Unlike pigs or poultry there are no UK commercial sheep-breeding companies.

In research, sheep are commonly used for antibody production, since they are easily handled and large quantities of serum (up to 500 ml) can be regularly harvested. They are also used for studies on fetal development, reproduction, endocrinology, respiratory function and orthopaedics, as well as a number of sheep diseases of economic and public health importance.

Behaviour

Sheep are very social animals that are normally docile and tractable. At pasture they spend a large part of the day grazing over wide areas, but they are timid and have very strong flocking instincts. If disturbed they rapidly flock together, which makes them relatively easy to drive. However, their well-developed flight reflex can easily turn into a headlong rush, in which injuries can occur. They will more readily move uphill, and away from dark towards light, and this should be borne in mind when designing handling facilities. Sheep become distressed if separated from others, and should never be housed singly, unless justified for experimental reasons, in which case they must always be within sight and sound of conspecifics. Entire males can be aggressive to humans.

Housing

Sheep are able to adapt to a number of different types of housing. They may be kept outdoors at pasture, or in covered or semi-covered yards, or in totally enclosed accommodation, which may or may not be environmentally controlled.

Their thick lanolin-impregnated fleeces make them well adapted to severe weather conditions and they can remain outdoors throughout the year in the UK, providing shelter from wind and driving rain is available. Fencing needs to be close-meshed and robust, approximately 1 m high, and free of sharp projections that may cause injury. Sheep do not usually jump over fences, but will test their strength by rubbing and pushing against them until they break. Outdoor housing has many environmental advantages for sheep, but they will be exposed to both internal and external parasites, and parasite-control programmes will be need to be drawn up. Young lambs may also be liable to predator attack (from dogs or foxes).

If sheep are kept indoors it is advised that they are kept in groups of no more than 30, matched by age and size to prevent bullying at feeding. Horned sheep should not be housed in the same enclosure as polled sheep.

The walls and doors of the enclosure should be non-porous and easy to clean. The lying area must have a solid floor, made of

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concrete or rammed chalk, which gives better drainage. Bedding should preferably be deep straw to provide insulation and comfort, although wood shavings or sawdust may be alternatives. Fresh, clean, dry bedding should be added on a regular basis, until the height of the bed requires its removal. The pen should then be washed down and allowed to dry before re-bedding.

Environmental enrichment can be difficult with sheep. Toys, such as balls or chains, do not seem to be of much interest to sheep. The provision of conspecifics, roughage and space to lie down to ruminate is probably the most important enrichment for housed sheep. Un-chopped root vegetables, such as sugar beet, are well liked and give them something to gnaw at. They may be provided as a treat on a daily basis, although in the long term there is a risk of damage to the incisor teeth.

Breeding ewes are usually housed in individual pens with their lambs for a few days at lambing time. This ensures that the lambs receive colostrum and develop the maternal bond. After 24–48 h ewes and lambs can be mixed with others in small groups.

Feeding

Sheep are obligate herbivores and their digestive tracts have adapted to a diet of vegetable matter. They have no upper incisor teeth, but in their place possess a hard, ridged fibrous 'dental pad', against which the lower incisors bite. They have no canine teeth, leaving a gap, the diastema, between the lower incisors and the molar arcades. The molars are continuously growing and adapted for grinding fibrous food. The jaw is articulated to allow movement from side to side for efficient chewing.

The non-glandular fore-stomach consists of three large chambers: the reticulum, the rumen and the omasum. The rumen and reticulum contain bacteria that digest cellulose, releasing fatty acids, and produce vitamins. Food from the rumen is regurgitated several times for extra chewing, in a process known as rumination. A side effect of the breakdown of cellulose is the production of large quantities of methane gas. This is removed from the rumen by frequent belching (eructation), which may occur as often as once every minute. If ruminants cannot release the gas produced, the methane will accumulate to distend the rumen, causing the condition commonly known as 'tympany' or 'bloat'. In severe cases the pressure of the distended rumen will cause circulatory disturbance and, unless relieved, death.

Food eventually passes from the rumen, through the omasum, which absorbs much of the fluid, to enter the abomasum, which is analogous to the glandular stomach of other mammals. In young lambs the rumen is undeveloped and milk passes via a muscular groove (the oesophageal groove) to enter the abomasum directly. The rumen gradually develops in response to the ingestion of roughage, and is fully functional by about 8 weeks of age. Sheep produce copious quantities of saliva, but do not vomit. However, drooling and spillage of regurgitated rumen contents may be seen.

Sheep are grazing animals and will nibble grass and vegetation at ground level. Under most circumstances they will obtain sufficient nutrients for growth and maintenance from grass alone. The optimum sward height for sheep is 2.5 cm, and they can have difficulty utilising very long grass. If the grass quality or availability declines sheep should be fed a long-fibre conserved forage, usually hay but alternatively silage or even straw. In some circumstances, such as pregnancy and lactation, the ewe's diet may require supplementation with higher energy 'concentrate' foods. Concentrates are normally based on cereals, with additional protein, vitamins and minerals. Approximately 125 g/head per day of a commercial sheep food should be fed daily from day 100 of pregnancy, gradually increasing to as much as 1 kg/head per day at parturition. Sheep fed on straw as their sole source of roughage overwinter, will require additional concentrate feeding.

Whenever sheep are kept indoors roughage should be permanently available in hayracks. If concentrate rations are also fed there must be sufficient room at the feed trough for all animals in the pen to feed at the same time. Where horned animals are kept additional space allowance must be made at the feed troughs to avoid injuries.

Mineral deficiencies may occur if the pasture or conserved foods are deficient in copper, cobalt or selenium. Supplementary minerals may be advised in these circumstances, but great care is required in their formulation, as sheep are peculiarly susceptible to copper toxicity. Concentrate rations or minerals intended for cattle or pigs contain added copper and should never be given to sheep, as they can induce copper toxicity.

Poor dentition can be a problem in older sheep. The incisor teeth are frequently lost. However, the majority of sheep can maintain satisfactory body condition without incisor teeth. Of more importance are the molar teeth, which are essential for masticating food. Loose or infected molar teeth are a common cause of weight loss in older sheep.

Growing sheep are particularly prone to urinary calculi when fed high levels of concentrate. In male lambs (entire or castrated) the urethra is narrow, and calculi may cause urethral blockage, with urine retention and fatal consequences. To prevent this it is essential that the magnesium and phosphorus content of concentrate rations be restricted. Salt licks should always be provided to encourage drinking and urinary throughput, and urinary acidifiers may also be helpful.

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Water

Sheep must be provided with clean water *ad libitum* at all times. They prefer to drink from flowing water, although this may be difficult to achieve in practice. Static water tanks are acceptable, but care is required because sheep can become cast in them and drown. Bowl drinkers are satisfactory in indoor pens, but nipple drinkers are not suitable for sheep.

Environment

Sheep will tolerate a very wide range of temperatures. The thermoregulatory neutral zone for sheep is 10–30°C, but the extremes of this range depend on whether they have a full fleece or not. Unshorn sheep in summer can suffer from heat stress, exhibiting panting and mouth breathing at temperatures over 20°C. This is exacerbated if they are kept indoors, and in these circumstances shearing should be undertaken in April or May, well in advance of any hot weather. Once shorn, sheep are more susceptible to cold and should not be exposed to temperatures of less than 10°C. If shearing is undertaken very early in the year they must be housed for at least 2 months before they are turned outside, to allow some fleece re-growth. Sheep permanently housed in environmentally controlled conditions will generally benefit from shearing twice a year, and should be kept at between 10 and 24°C. Adequate ventilation is essential to reduce the risk of respiratory disease. Extractor fans should remove 3 m^3 air/kg of body weight per hour to control ammonia levels and humidity levels should be kept between 45 and 65%.

Breeding

Sheep are seasonally polyoestrous, returning into oestrous every 16–17 days over the winter period (September–February). The onset of the breeding season is controlled by diminishing day length, but varies significantly between breeds. Normally the hill breeds start later (October–November) than the lowland breeds (August–September). Dorset sheep are unusual in having a very long breeding season, extending from about June to March. This means that they may be mated every 8 months, giving birth to three litters in 2 years. Dorset breeds and their crosses, particularly the prolific Finn-Dorset, are popular research animals.

Under farm conditions ewes are normally mated in the autumn, to give birth the following spring. They run with the rams (tups) for a period of about 6 weeks. Ultrasound scanning at 50–90 days of gestation is widely practised to determine pregnancy and count fetal numbers. Ewes carrying multiple fetuses can then be grouped separately for differential feeding.

Gestation lasts approximately 147 days (range 142–150 days), although there are slight breed differences. Ewes carrying multiples normally have shorter gestation times than those carrying single lambs, but as a rough guide, in the northern hemisphere, ewes mated on Guy Fawkes day (5 November) will lamb the following All Fools Day (1 April). Ewes may be mated as lambs (ewe-lambs) in their first autumn, at about 6 months of age, provided their body weight is at least 70% of the adult weight for the breed. Alternatively, they may be left until the second autumn, when they are 18 months of age. Weaning normally takes place at 12–16 weeks, although there are management systems where weaning may be as early as 6 weeks, provided suitable concentrate rations are provided.

In most sheep breeds the breeding season can be advanced by a number of methods. These include the use of progesterone-impregnated intra-vaginal sponges, melatonin implants or the use of vasectomised males. By advancing the breeding season in the UK to July or August lambs can be produced in December/January. Artificial insemination (AI) is possible in sheep using either fresh or frozen semen, and embryo transfer may also be carried out by laparoscopy under general or local anaesthesia.

Handling

Sheep are particularly easily startled and patience and gentle handling are required for success. They are inclined to follow one another, and will generally move away from humans, dogs or buildings towards open countryside. Where larger numbers are involved a proper sheep-handling system should be employed.

Where sheep are held indoors carefully designed housing, consisting of holding pens leading through narrow walkways and races to smaller pens, will allow individuals to be caught with minimal stress. Where such facilities do not exist the best method of restraint is to gradually pen the sheep into a corner using a number of hurdles. A trained handler can then restrain the individual sheep, by holding one arm under the neck and the other around the rump (see Figure 14.4). Sheep should never be caught by the wool, as grasping the fleece is painful and may result in handfuls of wool being torn out. It is also not advised that sheep be caught by their horns. It is an offence under welfare legislation to lift a sheep by its horns.

FIGURE 14.4 Restraining a sheep.

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To turn a sheep onto its rump (casting), the handler stands on the left side of the sheep, grasps the muzzle with the left hand, and pushes the head round to the right to face the tail. This can be facilitated by placing the thumb in the diastema behind the incisor teeth. With the right hand, press down on the rump and the hind legs will collapse. The sheep can then be rolled over and sat onto its rump (see Figure 14.5). Alternatively, with smaller sheep, the handler may be able to reach over and grasp the fold of skin low down on the right flank. Using this fold the sheep can be lifted up so that it comes to rest sitting on its rump.

FIGURE 14.5 Casting a sheep.

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Pain and stress recognition

Sheep in pain appear dull and depressed with little interest in their surroundings. They may become anorexic, have drooping ears and be reluctant to rise. However, sheep have a great ability to mask the signs of pain, and in the early stages these may only be apparent to an experienced stockperson. In the later stages separation from the group is a good indication that a sheep is sick. Nasal discharge is a common sign of respiratory infection, and grunting or teeth grinding usually indicate abdominal pain. Lameness is a common problem with sheep and should never be ignored. Animals are not lame unless they have pain in the limb.

Common diseases and health monitoring

Sheep are susceptible to a number of diseases, many of which are zoonoses. Very few commercial flocks have a well-defined health status, so it is safest to assume that all sheep are carrying a wide variety of sheep diseases and to screen any that are purchased for research purposes thoroughly. The rules for isolation and biosecurity outlined in the introduction to this section should always be followed.

Zoonoses

Of particular concern are the numerous zoonotic agents that may cause abortion in ewes (*Chlamydophilia*, *Toxoplasma, Campylobacter*, *Listeria*, *Coxiella* and *Salmonella*). For this reason it is advised that pregnant women do not handle ewes or lambs at lambing time. Contagious pustular dermatitis virus (Orf) is a common viral disease of sheep, causing facial lesions in lambs and a painful skin condition in humans. The protozoan parasite *Cryptosporidium parvum* may cause enteritis in lambs and humans.

Clinical diseases

Parasitic gastroenteritis (PGE) caused by gastrointestinal nematodes is a common and potentially fatal condition of lambs, and constitutes the major health threat to young, grazing sheep. Control can only be achieved by routine prophylactic anthelmintic treatment. However, increasingly worms are becoming resistant to many of the anthelmintics available. Veterinary advice should be sought on the selection and frequency of treatments. Sheep born and reared indoors are very unlikely to suffer from PGE.

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However, if grazing sheep are moved indoors they will almost certainly be carrying these parasites, and will need treating with an anthelmintic on housing. Liver fluke is another life-threatening parasite of sheep, associated particularly with grazing pastures prone to flooding.

Lice and psoroptic mange (sheep scab) cause irritation and wool loss in sheep. Treatment may be by contact chemicals or injection. Ticks and keds are important vectors of other diseases, particularly in the hill regions. Myiasis (fly strike) is seen in the summer and autumn months in the lowlands. Undetected, fly strike can prove fatal and prompt treatment is required.

Foot rot is a common infectious disease of sheep, caused by the bacterium *Dichelobacter nodosus*. It spreads rapidly when infected sheep are housed indoors, so prophylactic treatment at housing should be undertaken.

Clostridial diseases are common and nearly always fatal. These include diseases such as tetanus, and enterotoxaemias caused by *Clostridium perfringens*. Effective clostridial vaccines are commercially available and should always be used. *Mannheimia haemolytica* and *Pasteurella multocida* are common commensals found in the throat and nasal passages. Under stress, and particularly in conditions of poor ventilation and overcrowding, they can produce acute or chronic respiratory and systemic disease. Vaccines are available and should be routinely used in housed sheep. Other important bacterial conditions include *Corynebacterium pseudotuberculosis* (caseous lymphadenitis) and *Mycobacterium avium pseudotuberculosis* (Johne's disease).

Border disease virus (BDV) can produce infertility and fetal abnormalities. Maedi–Visna (M–V) and sheep pulmonary adenomatosis (SPA) are viral causes of chronic respiratory disease but may combine with *Ma. haemolytica* to produce acute disease.

Scrapie is a notifiable disease, and suspicion must be reported to DEFRA. It is slow-onset transmissible spongiform encephalopathy (TSE) of unknown aetiology, producing neurological changes in adult sheep. Resistance to scrapie is genetically controlled. The incidence of scrapie is now very low in the UK.

Anaesthesia and surgery

There are a number of difficulties to be addressed with general anaesthesia in sheep and these, together with their placid and stoical nature, have led to the widespread use of local anaesthetic techniques. The considerations below relate to adult sheep only. Lambs below 8 weeks of age (before the development of the rumen) can be treated as if possessing a single stomach.

Sheep are usually starved for up to 18 h and water withheld overnight before general anaesthesia to reduce the volume of the rumen contents and also reduce the amount of gas produced, particularly if the sheep has previously been at grass.

Sheep produce large quantities of saliva, which may pool in the pharynx during anaesthesia. Regurgitation of rumen contents is also possible. It is advisable to position the sheep with the head lowered and the nose pointing downwards, over the end of the operating table, to allow for drainage of these fluids by gravity. The use of drying agents such as atropine is generally contraindicated in ruminants, as it renders salivary and bronchial secretions more viscous and harder to clear. To prevent accidental inhalation of saliva or ruminal contents once the swallowing reflex has been lost it is also strongly advised that a cuffed endotracheal tube is inserted as soon as anaesthesia is induced. A laryngoscope may be needed for this.

Microbial activity in the rumen continues during anaesthesia, and can lead to a build-up of gases in the rumen, which becomes distended (tympany), interfering with surgical access to the abdomen, and putting pressure on the diaphragm, embarrassing respiration. This may be relieved by the use of a stomach tube. This results in the passage of large quantities of rumenal fluid, which should be collected into a bucket to avoid contamination of the operating theatre floor. Alternatively, a temporary rumen fistula can be created for the duration of the procedure.

If sheep are held on their back for long periods the weight of the rumen compresses the posterior vena cava, compromising venous return to the heart and leading to circulatory collapse. If possible sheep should be positioned with the hind quarters slightly lower, so that the weight of the abdominal contents does not rest on the diaphragm.

Table 9.3e gives suggested doses for anaesthetic and analgesic doses in ruminants. No analgesics are licensed for use in sheep in the UK, so those for other species should be used under veterinary direction.

The rumen occupies the whole of the left side of the abdomen. Abdominal surgery may take place through a mid-line ventral incision, but this requires the sheep to be in dorsal recumbence for a long period and, post-operatively, the weight of the rumen puts great tension on the suture line. For these reasons abdominal surgery is often conducted through an incision made in the left flank. Although this is directly over the rumen, this can be pushed aside to reveal the area of interest (uterus, liver, etc.). If incisions are made in the right flank, the pressure of the rumen can force intestines out of the wound, making it very difficult to operate.

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Useful data

Table 14.2 gives useful data for ruminants.

Table 14.2 Useful data: ruminants.

	Sheep	Goats	Cattle
Biological data			
Adult weight (kg), male		70–100	800–1000
Adult weight (kg), female		20–70	500–800
Natural lifespan (years)		9–18	15–20
Rectal temperature (°C)		38.5–39.0	38.5
Heart rate/min		60–135	40–100
Blood volume (ml/kg)		58–90	57–62
Respiratory rate/min		12–25	27–40
Breeding data			
Puberty (months)	7–12	3–8	12–15
Age to breed (months)	First or second autumn	Second autumn	15–24
Gestation (days)	144–150 (average 147)	147–155 (average 151)	274–291 (average 282)
Litter size	1–3	2–3	1–2
Birth weight (kg)	3–5	2–4	25–45
Weaning age (weeks)	12–20	12*	6*
Oestrous cycle (days)	16/17 (seasonal)	19/20 (seasonal)	21
Post-partum oestrus	No	No	Yes
Haematological data			
Red blood cells ($\times 10^6$/mm^3)	8–13	12–14	5–10
PCV (%)	24–40	24–48	24–46
Hb (g/dl)	8–16	8–14	8–15
White blood cells ($\times 10^3$/mm^3)	4–12	5–14	4–12
Neutrophils (%)	20–40	20–40	6–45
Lymphocytes (%)	40–70	50–65	18–75
Eosinophils (%)	0–15	3–8	2
Monocytes (%)	1–12	1–5	1–7
Basophils (%)	0–1	0–1	0–1
Biochemical data			
Serum protein (g/dl)	6.6–8.1	5.4–7.5	5.3–7.5
Albumin (g/dl)	2.4–3	2.7–4.6	2.1–3.6
Globulin (g/dl)	3.5–5.7	1.5–2.8	3–5.5
Glucose	100–500 mg/dl	75–150 mg/dl	2–3.2 mmol/l
Blood urea nitrogen	8–20 mg/dl	17–23.5 mg/dl	2–6.6 mmol/l
Creatinine	1.2–1.9 mg/dl	0.8–1.8 mg/dl	44–165 µmol/l
Total bilirubin	0.1–0.42 mg/dl	0.25–0.74 mg/dl	0–6.5 µmol/l
Cholesterol	52–76 mg/dl	35–53 mg/dl	1–3 mmol/l

*Offspring weaned at 4 days of age in dairy breeds.

Goats

The ancestor of the modern domestic goat (*Capra hircus*) is the Bezoar, found in the Middle East and Asia Minor. Selection for milk, meat or skins has led to the development of several breeds, of which the British Saanen, Toggenburg and Anglo-Nubian are the commonest in the UK. Some breeds, such as the Angora and Cashmere, have been developed specifically for fibre production

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(mohair and cashmere). Varieties of dwarf (pygmy) goats exist that weigh as little as 20 kg. Goats normally possess horns and may also have beards and wattles. The tail is naturally short and stands erect when the animal is alert. They have strong-smelling scent glands around the horns in both sexes and also under the tail in the male.

Behaviour

Goats are inquisitive animals and tend to be more independent than sheep. They normally live in groups, but will cope well if they have other species, such as humans or sheep, for companions. If disturbed they will scatter, relying on their agility over difficult terrain to escape. This makes them impossible to drive, unlike sheep. However, they can be led and will usually follow a handler, especially if they have food. They frequently stand on their hind feet to explore, and can easily reach to a height of 2 m, so this should be considered when suspending items near or above goat pens. Their curiosity causes them to lick or chew at any unusual materials, which can lead to the ingestion of foreign objects and may be the cause of accidental poisoning or even electrocution from suspended electric cables.

Goats form a strong social hierarchy and the introduction of new members to a group may result in fighting. This can lead to injuries because many breeds are horned. Males can be aggressive during the breeding season.

Housing

Goats are less well protected from extremes of cold than sheep and should always be provided with a roofed shelter3. They dislike rain and need a dry, draught-free bed. An open fronted building will usually be sufficient, provided it backs into the prevailing wind. Goats are particularly agile and can leap over barriers several feet high; so all fences should be at least 2 m.

Goats are natural climbers and the provision of a raised area in a pen, such a large straw bale, provides good environmental enrichment. However, they may also use such raised areas to jump out of the pen, so be careful not to site this too close to a fence. Owing to their natural inquisitiveness, goats will investigate objects, such as toys or chains, particularly if these are suspended above head height. The provision of roughage suspended above head height, to allow natural browsing behavior, is also recommended. Goats readily become accustomed to contact with humans, and this should be encouraged during routine husbandry procedures and at other times.

Feeding

Goats are primarily browsing, rather than grazing, animals and will strip vegetation off trees to a considerable height. However, in the absence of trees, goats will graze and can be maintained on grass in paddocks. As with other ruminants, conserved forage, such as hay or silage, must be fed during the winter months.

Lactating goats can produce as much milk per kilogram body weight as dairy cows and will normally require supplementary concentrate feeds. They are less sensitive to the toxic effects of copper in the diet than sheep.

Goats are fastidious eaters, and will refuse food that has been soiled by faeces or urine. Feeding troughs must be clean and hayracks should be suspended well above the floor. This not only prevents soiling of the hay but also allows the goats to perform their natural browsing behaviour.

Water

Goats are fussy drinkers and must be provided with clean water *ad libitum* at all times. Water bowls must be cleaned out regularly. Lactating goats may drink 10 litres or more of water daily.

Environment

Goats are more heat-tolerant than sheep, but less able to stand extremes of cold. When housed indoors they should be kept at temperatures between 10 and 24°C and at relative humidities of 45–65%.

Breeding

Like sheep, goats are seasonal breeders, commencing their oestrous cycles in the late summer/autumn and ending in mid-winter.

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Oestrus occurs approximately every 21 days throughout this period. Gestation length is 147–155 days and the litter size is normally between one and three. As with sheep it is possible to advance the breeding season with the use of hormones.

Pregnancy can be confirmed by ultrasound scanning at about 50 days of gestation. Some goats can develop pseudopregnancy, even if they have not been mated. They show increased abdominal and udder swelling over a period of months, which normally results in the eventual discharge of copious quantities of uterine fluid at the expected time of parturition. Such animals usually breed normally in future years. Goats are also unusual in that some will come into lactation without any prior pregnancy or parturition.

Puberty is reached at 5–8 months of age. AI and embryo transfer techniques are available. Kids are normally weaned at 5–6 months of age, although if the dam is producing milk for human consumption the kid will be artificially reared from 4 days of age.

Handling

Goats are usually horned, which can be dangerous for the handler. However, they are generally less nervous than sheep and can be caught quite easily with a little patience. They should not be caught or restrained by the horns, but by holding the hands either side of the head, behind the lower jaw. Unlike sheep, goats should not be restrained by casting, and all examinations should be performed in the standing position, as for a cow.

In order to facilitate future handling most goat kids have their horn buds removed at about 4 days of age (disbudding). If this procedure is delayed beyond a week there is a danger of incomplete removal and re-growth. This procedure must be carried out under general anaesthesia by a veterinary surgeon.

Pain and stress recognition

Goats are usually more vocal than sheep, although they too have an ability to mask the signs of pain. Dullness, drooping ears, reluctance to rise, anorexia, teeth grinding and separation from the group are all indicators of pain or distress.

Common diseases and health monitoring

Nearly all of the infectious diseases of sheep are transmissible to goats, as well as the majority of the sheep parasites. The major differences between the species can be summarised as follows.

- There are no licensed vaccines available for goats. However, some sheep vaccines may be used under veterinary direction.
- Goats are more susceptible than sheep to infections with mycobacteria (Johne's disease and tuberculosis, or TB). They should be routinely tested for TB, especially if they have been in contact with cattle.
- The Maedi–Visna virus of sheep produces arthritis, encephalitis, pneumonia and mastitis in goats, where it is known as caprine arthritis and encephalitis virus (CAE). This is more common in the USA than in the UK. Serological tests are available to detect infected animals.
- Goats do not usually suffer from fly strike, because their short-hair coat is kept cleaner than the long wool of sheep.

Anaesthesia and analgesia

The sedative, anaesthetic and analgesic agents recommended for sheep are also suitable for goats at identical dose rates (see Table 9.3e). Like sheep, goats are extremely sensitive to xylazine and this sedative should never be used.

Cattle

The domesticated cattle of the world are nearly all derived from two major species: *Bos taurus*, which includes all of the European breeds, and *Bos indicus*, which includes the Asian and African cattle. By selection, a number of different breeds have developed. Some of these are kept primarily for meat production (beef breeds) and some for milk production (dairy breeds).

In the UK, the Holstein–Friesian is the predominant dairy breed, although Jersey and Guernsey breeds remain in the Channel Islands and areas of the West Country. Holstein–Friesians are the largest dairy breed, weighing over 600 kg (bulls over 1000 kg), while Jerseys are the smallest (400 kg).

Beef breeds include the native Aberdeen Angus and Hereford, as well as continental breeds, such as the Charolais, Simmental and Limousin. Dairy cows are frequently mated to bulls of the beef breeds, to produce hybrid calves with greater growth potential

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and better carcass conformation than the pure Friesian.

Cattle are not widely used in research, because of their large size and expense. However, they are the species of choice for certain zoonotic infections (*Escherichia coli* 0157 and TB) and are also used for studies on ruminant physiology related to milk production.

Behaviour

Cattle are gregarious animals, and show clear signs of stress if isolated[4]. Wild cattle aggregate in groups of cows and calves, with bulls in separate groups. Bulls defend specific areas within the larger environment and occasionally interact with cow–calf groups. Affiliative behaviour includes allo-grooming, or social licking, often directed at the neck. Cows form grooming partnerships with specific individuals within a group.

Aggressive interactions occur when unfamiliar individuals are mixed together, but reduce as animals establish a dominance hierarchy. Aggression is more common in intact males than in castrated steers[5]. Aggressive behaviours include lowering the head and physical contact (head butting or head-to-head pushing). Bulls may also vocalise, or paw and rub the head on the ground.

Cattle show diurnal rhythms when on pasture. During the day they spend more time grazing, and at night more time lying down. Cattle also spend between 8 and 12 h lying down per day, in bouts lasting about 1 h. The diurnal pattern of feeding and lying down is highly synchronised when cattle are kept on pasture. This can be influenced by other factors, such as weather and access to shade. For example, cattle will spend more time standing and less time lying down in warm weather[6] and more time standing in shade during the day with more grazing at night to avoid the heat of the day[7].

Dairy cows are normally docile and creatures of habit. They rapidly become accustomed to the regular routines involved in twice daily milking and are habituated to close human contact.

Calves are easily handled up to about 12 weeks of age (100 kg), but as they grow their increasing weight can make them potentially dangerous to humans.

Beef breeds are generally less amenable than dairy breeds, because they are handled less regularly. All bulls are unpredictable and cause several human deaths each year. They must always be handled with extreme caution.

Housing

Cattle can survive outdoors in a very wide range of climates. However, it is customary to bring milking cows and most beef breeds inside for the winter months in the UK. During the grazing season (usually April–October in the south of England), cattle can be kept outside in fields enclosed by wire fencing approximately 1.2 m high.

Housed cattle are normally bedded on deep straw, over a base of concrete or rammed chalk. Part of the floor area (up to 20%) may be slatted to facilitate cleaning. Slatted floors are generally less welfare-friendly than solid floors and must be designed carefully. Groups should be matched by age and size and horned breeds should not be mixed with polled breeds to prevent injuries3.

Over winter, cattle may be housed in straw yards, cowsheds, kennels or cubicle systems. Cubicles may be bedded with straw, wood shavings, sand or lined with rubber mats. For ease of feeding dairy cows are normally grouped according to their date of calving and milk yield.

Environmental enrichment for cattle has not been well defined. Group housing, comfortable bedding and provision of roughage *ad libitum* are probably the most important aspects. The provision of toys and similar does not appear to elicit much interest.

Feeding

Beef breeds normally graze during the summer months and are fed conserved forage (hay, silage or straw) over winter. They receive little in the way of concentrate feeds and their calves are naturally weaned at 6–7 months of age. Dairy cows require additional concentrate rations throughout lactation, and the feeding of these to maximise milk yield has become a science in itself.

Dairy calves are normally removed from the dam at 2–4 days of age and reared on milk replacers. There are many different systems for artificially rearing calves, ranging from twice daily bucket feeding of warm milk, to fully automatic milk dispensers. Hay and concentrate rations should be made available after 2 weeks and weaning normally takes place at 6–7 weeks, at which time the calves should be eating in excess of 1 kg concentrate/head per day.

Indoor-reared cattle typically eat for 4–6 h/day, while on pasture cattle spend more time grazing, 6–10 h/day. In pasture

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systems there is space for all cows to feed at once. In barns, restricted space at the food trough make it is less likely that all cows will feed at the same time.

Environment

Calves artificially reared in calf houses are prone to enteric and respiratory diseases. They require temperatures above 15°C, a clean, dry bed of straw and good ventilation, without exposure to draughts.

Weaned calves housed indoors remain particularly prone to respiratory infections and good ventilation (either natural or forced) is essential. Temperatures should be above 10°C and humidity 45–70%. Wide fluctuations in temperature and humidity, as may occur in the autumn, can predispose cattle to pneumonia.

Adult cattle are able to withstand considerable variations in temperature and are normally kept at ambient temperature in sheltered buildings.

Breeding

Cattle are polyoestrous throughout the year, although they are generally less fertile during the winter months. The oestrous cycle lasts 21 days, but can be manipulated by the use of various hormones. Pregnancy can be confirmed by manual palpation per rectum, by ultrasound scanning or by a progesterone assay. Gestation length is 280 days (±10 days), although there are breed variations. Cows normally have a single calf, although twins are not uncommon. They will normally return to oestrus within 42 days of calving and are mated at approximately 85 days, to achieve a calving interval close to 365 days.

AI is practised widely in dairy cattle. Commercial AI centres hold stocks of frozen semen from genetically superior sires and supply a daily insemination service to farms. Embryo transfer is also commercially available for cattle, using stored, frozen embryos.

Handling

Cattle are generally docile and easily handled, but their large size makes a good handling system essential. In research establishments cattle may be trained to feed through a neck yoke, so that they can be restrained and haltered at any feeding time. A cattle crush is necessary to carry out examinations and procedures.

Calves less than 70 kg can usually be restrained by two handlers. One should hold the head, putting a hand across the bridge of the nose (taking care not to obstruct the nares), and turning the head towards them, while the other restrains the rear end. For cattle over 100 kg a halter should be used to restrain the head or the animal placed in a neck yoke or crush (see Figure 14.6).

FIGURE 14.6 Restraint of a cow for blood sampling.

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Pain and stress recognition

Cattle show the same signs of pain as sheep and goats. They appear dull and depressed with little interest in their surroundings. They may have drooping ears, show a reluctance to rise and show anorexia. In later stages separation from the group and grunting or teeth grinding may be heard.

Common diseases and health monitoring

Zoonoses

Cattle suffer from several important zoonoses that may be fatal in humans.

- Bovine spongiform encephalopathy (BSE) was common in adult cattle from 1985 to 2000, but has now been eradicated from the UK.
- Regular skin testing for *Mycobacterium bovis*, the causative agent of bovine TB, is mandatory under DEFRA. It is recommended that cattle should be tested for this additionally before introduction to a research establishment. *Mycobacterium avium paratuberculosis* (avian TB) is seen occasionally in adult cattle over 2 years of age.
- Salmonellosis is a common cause of diarrhoea in neonatal calves, particularly if they have been traded through a market. All purchased calves should be tested on arrival.
- *Leptospira hardjo* can be carried asymptomatically by cattle and transmitted to humans via splashes of infected urine. A vaccine is available and should be used routinely.
- *E. coli* is common in calves, and may be asymptomatic. However *E. coli* 0157 may be fatal to humans.
- *Brucella abortus* has been eradicated from the UK but remains an important zoonosis in many parts of the world.
- Calves frequently suffer from 'ringworm', a fungal skin disease that is commonly transmitted from cattle to handlers.

Clinical diseases of cattle

Parasitic infections are less important in cattle than sheep, although young calves may be badly affected. Liver fluke, seen when ruminants graze pastures liable to flood, can cause severe disease in both sheep and cattle of all ages. Lungworm, due to *Dictyocaulus viviparus*, can cause pneumonia in all ages of cattle, but there is a vaccine available. Coccidiosis may be problematic

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in young calves, especially when reared indoors.

Three respiratory viruses are common in cattle: bovine respiratory syncytial virus (BRSV), parainfluenza 3 virus (PI3), and herpesvirus 1 (infectious bovine rhinotracheitis, IBR). They often produce disease in association with bacterial agents, such as *Ma. haemolytica*, *M. bovis* and *Haemophilus somnus*.

Rotavirus and coronavirus infections are also common causes of diarrhoea in neonatal calves. Bovine virus diarrhoea (BVD) is very common, but clinical signs are usually mild with complete recovery. However, if infected during pregnancy the fetus may be aborted, or mutated, or if the calf is born alive it may be persistently infected with the virus. Persistently infected calves are immunosuppressed and are unsuitable for use in research. They also act as a source of BVD infection for other cattle and will cause severe fertility problems in a herd. All purchased cattle should be tested for their BVD status by blood sample before arrival.

Cattle may suffer from lice infestations, but do not generally suffer from fly strike.

Anaesthesia

By virtue of their placid nature and large size cattle are particularly suited to regional nerve blocks, such as epidural and paravertebral. If general anaesthesia is given the precautions given above for sheep and goats apply. Drug doses are listed in Table 9.3e.

In adult cows the endotracheal tube may be introduced into the trachea by inserting a hand into the back of the mouth and guiding it into the larynx. A gag should always be in position between the molar arcades to prevent accidental closure of the mouth on the operator's arm during this procedure.

Useful data

Useful data for cattle are listed in Table 14.2.

Pigs

The various breeds of domestic pig are originally derived from the European wild pig, *Sus scrofa*, probably crossed with fast-growing Asiatic pigs such as *Sus vittatus* and *Sus indicus*. Pure breeds include the Large White, the Landrace, the Saddleback, the Duroc and the Pietrain. However, the majority of commercial breeding pigs are hybrids. These are produced by a number of UK pig-breeding companies and have been developed for high fecundity and the rapid growth of their offspring. Adult sows can weigh up to 250 kg, whereas boars are over 300 kg.

Miniature pigs (mini-pigs) are much smaller in size. Sows of the Yucatan and Göttingen breeds only reach 50–70 kg as adults, which makes them easier to house and manage in a laboratory environment.

Supply

Pigs may be obtained from any supplier. Mini-pigs can be purchased from specialised commercial breeders of laboratory animals. Commercial pigs are best sourced from pig-breeding companies, who maintain herds free from many of the major pig pathogens. It is also possible to source pigs from local farms, but their health status is likely to be less well defined. It is sensible to obtain pigs from clean suppliers when these can be identified. In any event, pigs should be quarantined and faecal swabbing and culture for pathogens such as *Salmonella* should be undertaken before studies are commenced. It is wise to source pigs from farms that are free from diseases, or where the pigs have been vaccinated.

Behaviour

Pigs are lively, intelligent, gregarious animals that are usually friendly to humans, although newly farrowed sows can be very protective of their litters and adult boars should always be treated with extreme caution. They should be group housed wherever possible, and in a stable group they will develop a dominance hierarchy. If unfamiliar animals are placed together they will normally fight and individual pigs should not be added to a stable group, as they will certainly be bullied and may be killed. It is always best to start with large groups and reduce these in numbers as the pigs grow. However, if mixing is unavoidable then it is

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better to carry this out on neutral territory at feeding time or at dusk. A tranquillizer, such as azaperone, may be used prior to mixing, in extreme cases.

Pigs rapidly become bored, and a barren environment will lead to stereotypic behaviour patterns, such as ear or tail biting, flank sucking or bar-biting. These vices may be minimised by environmental enrichment in the pen. Pigs have a very keen sense of smell and also well-developed rooting behaviour. If housed outside they will rapidly turn any pasture into a mud-bath by rooting for worms.

Housing

The majority of pigs used in research are kept indoors, either in naturally ventilated or controlled environment buildings. Good building design will encourage cleanliness, as will the provision of suitable bedding. Contrary to popular belief pigs are naturally clean animals, and if provided with sufficient space will defecate only in the coolest, wettest areas, which can then be scraped out or hosed down daily, leaving their bedding area clean and dry. The lying area of the pen should be solid and insulated. It should have bedding, such as sawdust, wood shavings or straw, to provide warmth, comfort and a substrate for rooting behaviour. Pigs will chew and play with straw, aiding digestion and reducing boredom. If no bedding is provided the ambient temperature must be higher and it is likely that more leg injuries will occur. The part of the floor where the pigs are fed and watered should remain un-bedded, and the pigs will use this area for defecating.

Farrowing sows require special accommodation. There is a huge size difference between the sow and her piglets, and with litters of up to 16 it is not uncommon for a sow to inadvertently lie on one or more of her piglets. Farrowing crates and other similar devices designed to reduce this risk are available, and may be used to house the sow from parturition until the piglets are weaned at 4 weeks of age (see Figure 14.7). However these may not be welfare friendly. Newborn piglets require a temperature of 30–32°C, whereas the sow prefers a temperature of 18°C or lower. By restraining the sow in a farrowing crate a heated 'creep' area can be provided within the pen to attract the piglets away from the sow when they are not feeding. This creep area should contain a heat lamp or heat pad and a special 'creep' feeder.

FIGURE 14.7 Sow in farrowing crate.



Pigs easily become bored and can develop stereotypic behaviour when confined in pens. Environmental enrichment must be provided for indoor pigs and a number of different devices are available, such as heavy-duty plastic footballs, suspended chains and a variety of plastic playthings. Feed-balls are available, which distribute the daily feed ration only if the pig rolls the ball, which encourages activity and exploratory behaviour. Cheap but effective enrichment can be obtained by throwing empty feed-

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bags into the pen, when the pigs will spend some time tearing these to shreds. Pigs should be group housed where possible, but stable groups are essential to stop fighting. The provision of deep bedding encourages rooting behaviour. In hot weather, a cool shower from a hose tied high above a corner of the pen can provide entertainment and prevent heat stress.

Feeding

Pigs are omnivorous single-stomached animals that will eat a variety of foods. The vast majority of pigs are fed commercial diets, manufactured from ground cereals with added protein, vitamins and minerals. These diets vary according to the age and physiological requirements of the pig. Young pigs are normally fed high-protein pellets, whereas growing and adult pigs are fed restricted amounts of lower protein nuts. Pigs may also be fed directly from the floor in a clean environment, which has the benefit of allowing them to express natural rooting behaviour (see Figure 14.8). The concentrate food may also be purchased as a finely ground, unpelleted meal and fed with water in troughs as gruel.

FIGURE 14.8 Pig rooting for food in straw bedding.



Pregnant sows require a ration containing 12–14% crude protein and will eat 1.8–2.3 kg daily, rising to 2.7 kg immediately before farrowing. Lactating sows should receive a 16–18% protein ration and may eat up to 10 kg daily. Piglets will begin to eat creep feed from about 10 days of age, but it must be fresh to be palatable. Only offer small amounts to start with and clean out uneaten food daily. Creep feed usually has a protein level of over 20%.

Following weaning at 4 weeks of age, creep continues to be fed *ad libitum* until the piglets reach about 6 weeks. After this, lower-protein feeds are normally given *ad libitum* until 12 weeks, following which the feed intake is restricted.

Mini-pigs have not been selected for any of the production characteristics of commercial hybrids (rapid growth, high lean/fat ratio and high fecundity), and therefore have different feed requirements in terms of both quantity and quality. In particular they are prone to excess fat if fed *ad libitum*, and care must be taken not to overfeed them. Special commercial diets for mini-pigs are available.

Water

Water is particularly important for pigs, and must be available at all times. Water deprivation, even for short periods, will cause a rapid increase in blood sodium levels (salt poisoning), which can be fatal. Automatic watering systems are best as they provide water *ad libitum*. This may be delivered in troughs or bowls (which tend to become dirty) or nipple drinkers (which are much cleaner). Troughs and drinkers need to be above ground to prevent contamination with faecal matter. Buckets and loose troughs are not suitable for pigs as they play with them and soon knock them over. As a general rule adult pigs require 1 litre/10 kg of body

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weight per day, whereas growing pigs and lactating sows need up to twice this amount. However, these figures are heavily dependent on temperature and feed, so water should always be provided *ad libitum*.

Environment

Pigs have very few sweat glands and find it difficult to lose heat in hot weather, leading to heat stress, which can sometimes be fatal. Indoor facilities without a cooling plant may find it difficult to prevent overheating in the height of summer. In these buildings cold water from a hose should be sprayed over the pigs if they show signs of heat stress. This is also a form of environmental enrichment, as pigs love to play under a shower of cool water. Pigs directly exposed to the sun can also suffer from sunburn, and shade should be provided in the summer months. Outdoor pigs will make good use of mud wallows.

Although pigs have only a sparse hair covering, their subcutaneous fat deposits help them to keep warm. The optimum temperature for adults is 16°C, which is easily achieved providing the accommodation is well insulated and they have deep-straw bedding and shelter from draughts.

Piglets have a high surface area to body weight ratio and so need high ambient temperatures (28–32°C) for the first few weeks of life. The temperature should gradually be reduced as the pigs age, dropping to 16°C when the pig reaches 80 kg (for Large White pigs).

Relative humidity should be kept between 45 and 65%, but adequate ventilation must be supplied to reduce the levels of ammonia and pathogens in the air, and to dissipate the large amount of heat produced by the pigs. Air speed in lying areas should not exceed 0.2–0.3 m/s for adults and 0.1 m/s for piglets, or excessive cooling occurs.

Breeding

Pigs are prolific breeders and will breed all year round. Large White pigs reach puberty at around 6 months of age and are normally bred from 8 months at about 180 kg. Maiden gilts can be triggered into oestrus by boar contact from 160 days of age.

The oestrous cycle lasts 19–23 days (average 21 days). Oestrus can be detected by pressing down on the sow's back – the back pressure test – when she will stand to be mounted if in ‘heat’. Other signs of oestrus are vulval swelling and vocalisation. Pregnancy may be confirmed by ultrasound scanning from 23 days of gestation. Pregnant sows are normally group housed until 7 days before farrowing, when they are moved into the farrowing crates.

Gestation lasts 114 days, and litter sizes vary between 3 and 18 piglets (average 10.5), dependent on the breed and age of the sow. Litter sizes are maximal for the fifth, sixth and seventh litters and then decline. Prolific commercial sows can produce up to 2.4 litters and rear more than 25 piglets each year. Piglets are weaned from 4–5 weeks. Sows normally return to oestrus 4–7 days following weaning.

Mini-pigs reach puberty at about 4 months of age and have an oestrous cycle of 19–20 days. They have smaller litters than commercial pigs (average of five piglets) and the piglets seldom weigh more than 500 g. Weaning tends to be later (5–6 weeks). The inbred nature of these pigs, coupled with their tendency to become overweight, can lead to infertility problems.

Growth

It is important for piglets to suck colostrum from the sow within 24 h, as no antibodies cross the placenta and intestinal closure takes place after 24 h. If piglets are colostrum-deprived they gain no maternal immunity and are prone to diarrhoea and other diseases.

Piglets are born with minimal body-fat reserves and need to suck frequently in the first 48 h of life to prevent the development of hypoglycaemia. Piglets born indoors, without access to soil, are prone to develop iron-deficiency anaemia and should receive an injection of an iron preparation within the first few days of life. The offspring of commercial hybrids grow very rapidly and should weigh 7–8 kg when weaned. Daily weight gain following weaning is highly dependent on feeding and genotype, but can reach as much as 1 kg/day.

Mini-pigs have similar requirements to standard pigs, but grow more slowly, only reaching 50 kg by about 12 months of age.

Handling

Pigs are easily startled and so should always be approached patiently and quietly. Handling and restraining pigs is usually accompanied by loud squealing, which ceases only when the hold is released. Force should not be used to move pigs, since this

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will alarm them and cause them to become noisy, obstinate and potentially dangerous. However, they are curious animals and naturally explore their environment. This can be exploited, since they will move along passages quite freely, and a rigid pig board can be used to guide them. Pigs will turn away from solid surfaces (see Figure 14.9). Large pigs may be guided into a crate, or if this is not available they can be restrained by using a rope snare (snatch), placed around the upper jaw behind the canine teeth. The pig pulls away from the snatch, causing the rope to tighten over the nose. Small piglets may be picked up, providing the abdomen is always supported. They should not be picked up by the hind legs alone. Larger piglets can be tucked under one arm, with the weight supported by the other arm placed under the belly. A foreleg can be held to provide additional restraint.

FIGURE 14.9 Moving a pig using a pig board.



Pain and stress recognition

Pigs in pain will show a decrease in their normal inquisitive behaviour, becoming listless and quiet. However, they may show aggression on being handled, so care should be taken. In white pigs the skin may show marked colour changes (pallor or cyanosis) and there may be piloerection of the sparse hair coat. In a group the sick pig will fail to thrive and may develop abnormal behaviour patterns, such as urine sucking.

Common diseases and health monitoring

Pigs are prone to a number of diseases (Table 14.3), some of which are notifiable under the UK Animal Health Act 1981, such as classical swine fever (CSF) and foot and mouth disease (FMD). Purchasing from sources of defined health status, using tailored vaccination programmes and applying rigid biosecurity measures, will control most pig diseases. The Federation of Laboratory Animal Science Associations (FELASA)8 has published recommendations for health monitoring programmes in experimental pigs.

Table 14.3 Diseases of pigs.

Disease	Comments
Zoonoses	
Salmonella species	Subclinical infections in many herds. Monitoring is conducted on pigs at abattoirs.

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Streptococcus suis Type 2	Carried asymptomatically in the oral cavity. Causes meningitis under certain circumstances. Classified as a zoonosis, but transmission to humans is rare.
Influenza virus	Various strains of influenza A endemic in most UK pig herds. Usually mild in pigs, but can be serious. Transmission to humans is possible, but rarely results in disease. New strains appear occasionally, which cause pandemic disease.
Erysipelothrix rhusiopathiae (erysipelas)*	Common cause of systemic disease in pigs. Can also cause skin disease in humans.
Clinical diseases	
*Mycoplasma hyopneumonia**	Causes enzootic pneumonia (EP). Widespread in UK pig herds. Most commercial herds vaccinate piglets.
Actinobacillus pleuropneumoniae (APP)*	Common. Protected against by vaccination.
Toxigenic *Pasteurella multocida**	Causes atrophic rhinitis (AR). Common. Protected against by vaccination.
Brachyspira hyodysenteriae	Swine dysentery. Seen in older pigs.
*Clostridium** and *E. coli**	Neo-natal diarrhoea
Enteric coronavirus	Transmissible gastroenteritis (TGE)
Porcine parvovirus*	Affects fertility
Porcine reproductive and respiratory syndrome*	Affects fertility
Leptospirosis	Many pig herds serologically positive, but few exhibit any clinical signs of infection.
*Haemophilus parasuis**	Causes Glässers disease.
Circovirus 2	Post-weaning multisystemic wasting syndrome (PMWS). Affects growing pigs 10 weeks or older. Virus causes immunosuppression, so previously subclinical diseases become clinical. Endemic in UK pig herds and sourcing pigs free of PMWS is very difficult. Mortality can be high.
Endoparasites	Uncommon in indoor pigs.
Ectoparasites	E.g. mange mites and lice. Can cause skin irritation and self-mutilation
*Vaccines are licensed for use in pigs to protect against these diseases.	

Techniques

Intravenous injections

Pigs lack easily accessible superficial veins and so intravenous injections can be difficult, especially in very small piglets. The marginal ear vein is the vein of choice, as the jugular vein is too deep to be certain of needle placement. Local anaesthetic cream (e.g. EMLA, AstraZeneca) can be applied over the site of the ear vein 15 min before injection, to desensitise the skin. Less pain is caused if flexible cannulae are used, rather than rigid needles.

Intramuscular administration

Care must be taken if giving intramuscular injections that the agent is not injected into the thick subcutaneous fat layer by mistake, or absorption of the drug will be delayed and the expected effect will not be achieved. The usual site for i.m. injections is the musculature of the neck and an 18 G, 40 mm needle should be used in adult pigs. For ease of administration the needle may be attached to the syringe by a long, flexible extension line to avoid the need for prolonged restraint.

Subcutaneous administration

Since pigs have a thick layer of subcutaneous fat immediately below the skin, subcutaneous injections in most places may simply end up in the fat layer. Subcutaneous injections may be given into the skin at the base of the ear, or into the fold of skin

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immediately in front of the hind leg.

Anaesthesia

Anaesthesia in the pig is generally straightforward, but there are some unusual features that should be borne in mind. The most satisfactory method of inducing anaesthesia is to administer an injectable anaesthetic agent intravenously, which is facilitated by prior sedation. Doses for anaesthetic and analgesic drugs can be found in Table 9.3f.

Food should be withheld for 8–12 h before anaesthesia to prevent vomiting (1–3 h in neonates). Water must be available at all times up until an hour before the anaesthetic is given.

Respiratory depression and/or apnoea are common complications of surgical anaesthesia in the adult pig, particularly if it is very fat. The head should be kept at as natural an angle as possible to prevent pressure on the larynx. If the pig is not intubated it is essential to ensure that the airway is kept clear and laryngospasm is prevented. Applying pressure on the vertical ramus of the mandible to push the jaw forward, and extruding the tongue, will help to prevent obstruction. Administration of atropine (0.3–2.4 mg depending on the size of the pig) to dry secretions will also help.

Inhalation agents (isoflurane or sevoflurane) are ideal for maintaining anaesthesia in pigs, following induction with injectable agents. Endotracheal intubation is straightforward in young pigs, but can be difficult in larger pigs. A larnygoscope with a very long blade is required to visualise the larynx in adults. The pig trachea is narrower than might be expected for an animal of its size and there is also a pharyngeal diverticulum, which can cause confusion. It is common to intubate adult pigs in dorsal recumbence, but intubation in ventral recumbence is also possible. The tube needs to be long and a rigid stylet is very helpful.

Pigs have poor thermoregulation mechanisms and can develop hyperthermia during prolonged anaesthesia. Some strains of pig also have an inherited biochemical myopathy known as porcine malignant hyperthermia. In these pigs, when anaesthesia is induced, a contracture of the skeletal muscles occurs, the pig becomes rigid and its temperature begins to rise. The strains of pig most commonly affected are the Poland–China, the Pietrain and some lines of Landrace and Large White. However, genotyping has allowed breeding companies to select against this trait and they will normally be able to supply pigs free of this condition, if requested.

Useful data

Useful data for pigs are found in Table 14.4.

Table 14.4 Useful data: pigs.

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	Large White	Both species	Yucatan
Biological data			
Adult weight (kg)	220–250		70–80
Food intake	Needs vary with production status		
Water intake	1 l/10 kg		
Natural lifespan (years)	16–18		8
Temperature (°C)	38.7–39.7		38.7–39.7
Heart rate/min	50–100		50–100
Blood pressure systole (mmHg)	150		150
Blood volume (ml/kg)	56–69		56–69
Respiratory rate/min	10–16		10–16
Breeding data			
Age at puberty (months)	5–9 (average 7)		4
Age to breed (months)			
Male	9		5
Female	After third oestrus		5
Gestation (days)	113–115 (average 114)		113–114
Litter size	8–12		6
Birth weight (kg)	1.3		0.56
Weaning age	4–5 weeks		5–6 weeks
Oestrous cycle	19–23 days (average 21)		19.5 days
Haematological data			
Red blood cells ($\times 10^6$/mm^3)	5–7		5–10
PCV (%)	32–45		22–40
Hb (g/dl)		10–16	
White blood cells ($\times 10^3$/mm^3)	7–20		4–10
Neutrophils (%)		30–50	
Lymphocytes (%)		40–60	
Eosinophils (%)		0–10	
Monocytes (%)		2–10	
Basophils (%)		0–1	
Platelets ($\times 10^3$/mm^3)		350–700	
Biochemical data			
Serum protein (g/dl)		6–8.9	
Albumin (g/dl)		1.6–3.8	
Globulin (g/dl)		5.2–6.4	
Glucose (mg/dl)		100–500	
Blood urea nitrogen (ml/dl)		10–30	
Creatinine (mg/dl)		1–2.7	
Total bilirubin (mg/dl)		0–0.6	
Cholesterol (mg/dl)		36–54	

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Chapter 15

Wild Animals

General Considerations

Research using wildlife species is often done for animal welfare and conservation reasons but field studies carry multiple risks for the animals involved and Refinement needs to be applied as rigorously as for other areas of research using animals[1]. There are some particular considerations when dealing with wild animals. The work is often unpredictable (such as the trapping of non-target species) and there should be appropriate contingency planning that identifies and mitigates the risks.

Since wild animals are not used to being handled they are likely to be more stressed by the presence of humans and by procedures than standard laboratory species. Captivity will induce particular stresses, and in many cases their exact biological needs will be unknown. All these features will contribute to the overall welfare burden on such animals and this should be taken into account when performing the harm/benefit analysis on the project licence application. Researchers should familiarise themselves in advance with the needs of the particular species, and techniques to minimise the distress caused to the animals during captivity. In addition to the physiological effects of handling stress on wild animals, they may also suffer from injuries during capture ranging from minor effects such as tooth and claw damage to severe injuries such as broken limbs or myopathy. The symptoms of myopathy have a delayed onset and so may not be seen at the time of capture but effect the animal after it has been released back to the wild. It is therefore very important to keep stress to the animal as low as possible by good handling and only brief periods of pursuit, capture and restraint.

The health status of animals in the wild will be largely unknown so adequate precautions should be taken to reduce the spread of infection and to protect personnel from the potential risk of exposure to zoonotic agents.

Legislation and Guidance

In the UK many wild species will be covered by other legislation in addition to the Animals (Scientific Procedures) Act 1986 (ASPA; see Chapter 2). Wild animals are protected by the Wildlife and Countryside Act 1981 (WCA). Part 1 of the WCA protects all wild birds and protected animals, which includes some mammals (all species of bat and species of dolphin, porpoise and otter), amphibians, reptiles and many species of insect. Under this Act it is an offence to take, kill, injure or sell a protected species, to disturb a protected species in its nest or place of shelter and to possess a protected species. To carry out such an activity therefore requires a licence issued by the appropriate authority. Some endangered or threatened species are covered by the Convention on International Trade in Endangered Species of Flora and Fauna (CITES; www.cites.org), which may affect the international movement of samples collected for scientific purposes. For work done in the UK it will also be necessary to take account of the following:

- the Animal Welfare Act 2006,
- Conservation (Natural Habitats etc.) Regulations 1994,
- Wild Mammals Protection Act 1996,
- and a range of species-specific legislation (e.g. the Protection of Badgers Act 1992, the Deer Act 1991)

Since there is such a wide variety of legislation that also varies between England and Wales, and Scotland and Northern Ireland according to devolved systems of government, a complete up-to-date guide is impossible to provide in this text. The UK statute law database (www.statutelaw.gov.uk) should be consulted for further guidance[2]. A useful summary of the relevant legislation covering keeping of wild animals in captivity can be found at www.defra.gov.uk/wildlife-pets/.

Relevant professional or local guidance must be followed at all times. This will include, as appropriate:

- guidance from expert bodies (such as the British Trust for Ornithology (www.bto.org) or Nature Conservancy),
- codes of practice set by professional bodies and learned societies (e.g. Association of the Study of Animal Behaviour

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(www.elsevier.com/framework_products/promis_misc/ASAB2006.pdf), Mammal Society, British Association for Shooting and Conservation (www.basc.org.uk) and English Nature (www.english-nature.org.uk),
- guidance from site owners/wardens,
- guidance from sponsors,
- health and safety advice,
- welfare guidance.

No one may work with animals in any capacity without having satisfactorily completed appropriate training. For example, if licences are required under the Animals (Scientific Procedures) Act, licensee training will be needed. In the case of research on wild birds training will be required in the netting and ringing of birds (from the British Trust for Ornithology), and for the trapping of mammals training from the Mammal Society may be needed, and training in relevant health and safety issues may be necessary. The supervisor of the programme must ensure competence in any necessary handling of the species, and the researcher must be sufficiently familiar with the habits and biology of the species to detect major departures from the normal state. Veterinary or animal welfare advice must be sought and taken in any case where the health or welfare of an animal gives rise to concern. Adequate time must be allocated to allow aptitude to be developed with adequate training, then assessed and the required standards achieved. For netting birds 6 months training is typically advised.

Considerations under the UK Animals (Scientific Procedures) Act 1986

Any scientific procedures in wild animals that may have potential to cause pain, suffering, distress, or lasting harm will be regulated under ASPA and may only be carried out as approved. The Home Office can advise on a case-by-case basis and must be consulted wherever a question arises as to whether or not a particular programme of work requires regulation under ASPA. Capture and handling are not in themselves regarded as coming within the definition of a regulated procedure but best practice is essential to ensure that trapping does not cause pain, suffering, distress or lasting harm. The netting and ringing of birds is also not regulated under ASPA, but is covered by controls drawn up by the British Trust for Ornithology on behalf of the Joint Nature Conservancy Council, English Nature and other bodies, under the WCA. Anyone wishing to work in an area which requires the capture of wild birds must be familiar with these controls and have received the necessary training.

It is important to bear in mind the potential consequences which interventions may have on animals other than those captured; for example on dependent young (lactating females should be promptly released from traps). Relevant issues should be discussed with the Home Office Inspectorate because regulation under ASPA may be required if a project has potential to cause distress to, for example, animals dependent on those captured.

Project Planning

Before beginning a project involving the use of wild species, there must be careful planning with attention paid to contingency and risk assessment. Consider the following points.

- Consult a statistician at an early stage to ensure that the minimum number of animals consistent with the aims of the project is used, and that animals are not wasted by having a sample that is too small. This is of course true for all projects involving animals, but is particularly important when dealing with endangered or threatened species.
- Review the literature, to become familiar with current techniques and husbandry methods. Ignorance of these may cause undue distress and is unacceptable.
- Ensure familiarity with the biology of the species involved. Avoid disturbances at times when interference may have a great impact on the species as a whole, such as breeding times, nesting, late pregnancy, parturition and neonatal periods.
- Avoid causing disturbances in the area to other species that are not being studied, and minimise damage to the habitat.
- Ensure maximum use of samples and material obtained from the study.

Catching and Trapping

The trapping of wild animals for use in research should be humane and may only be undertaken by competent personnel. The trap must keep the animal alive, uninjured and in a comfortable microenvironment. An open-sided wire mesh trap is unlikely to fulfil this requirement, since the animals will be uncomfortable and may feel exposed and vulnerable. Traps should be designed

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according to the biology of the species, and should avoid trapping non-target species. Best practice will include careful attention to the choice of trap, its layout, siting and positioning; and appropriate supply of food and bedding. Consider the animal's natural circadian activities: nocturnal animals should be held in darkness during daylight hours by covering the traps, and animals held in traps without food bait should be released so that they have sufficient time to gather food after release, or given supplementary food and water before release. Extra care will be needed in hot or cold weather or where traps may be in the presence of predators. Traps must be checked sufficiently frequently that stress to captured animals is minimised. Equal care is required on release to ensure animals have not been unduly compromised in their chances of survival. Consideration must be given to their ability to re-establish themselves within their own community and to find sources of food and water and that they are not unreasonably exposed to predators.

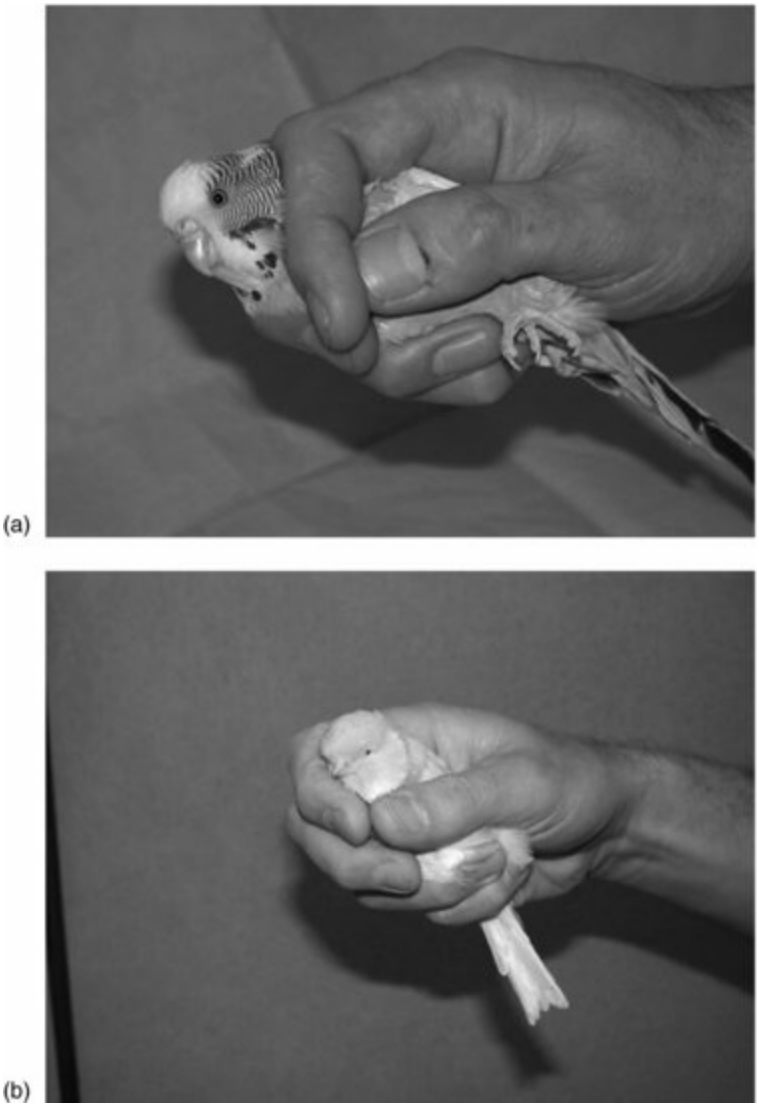
Handling Wild Animals

The amount of handling should be kept to the absolute minimum to reduce stress and also to minimise the risks to the handler. Wild animals are likely to bite, scratch or otherwise injure the handler, their health status is unknown and they may be carrying potentially zoonotic diseases (such as *Leptospira*, *Crytosporidium*)[3], so appropriate protective clothing should be worn and risk assessments carried out. For many species, keeping the animal covered will aid handling and birds should be handled by appropriate restraint of the wings. All personnel working with wild animals should be appropriately trained in their care, and familiar with the action to be taken in the event of injuries, escape or disease. It is important to develop a strategy for dealing with injuries that are due to the procedure, or which the animals sustained prior to capture, so that decisions over whether or not to treat or euthanase such animals can be defended[4,5].

Birds are very susceptible to stress, particularly small species, and excessive exertion can result in respiratory collapse or hypoglycaemic episodes. These problems can be reduced by handling them slowly and deliberately, and by ensuring that movements of the sternum are not restricted. Injuries may occur as the birds flap their wings to try to escape, so gentle restraint of the wings is vital. Small birds can be held with the back against the palm of one hand. Place the thumb and index finger or index and middle fingers each side of the head to prevent the bird from turning and pecking the handler, and wrap the other fingers around the body to restrain the wings and legs (Figure 15.1). All birds are disoriented by sudden darkness, so handling can be facilitated by covering the head with a cloth or bag, or for a small bird with a yoghurt pot. Large birds should generally be approached from above, with the base of the wings held in a naturally folded position without flapping. Gloves, tissue or a cotton cloth should be used to prevent the feathers coming into contact with perspiration from the hand, since this can reduce waterproofing and interfere with insulation. Assistance may be required to restrain the heads of large birds with long necks[6]. Because of the potential health risks from handling birds, handlers should wear appropriate protective clothing, including gloves and a face mask as a minimum, to handle birds. Cuts and grazes should be protected, and hands and arms washed thoroughly in disinfectant afterwards.

FIGURE 15.1 Restraint of a small bird.

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Anaesthesia of Wild Animals

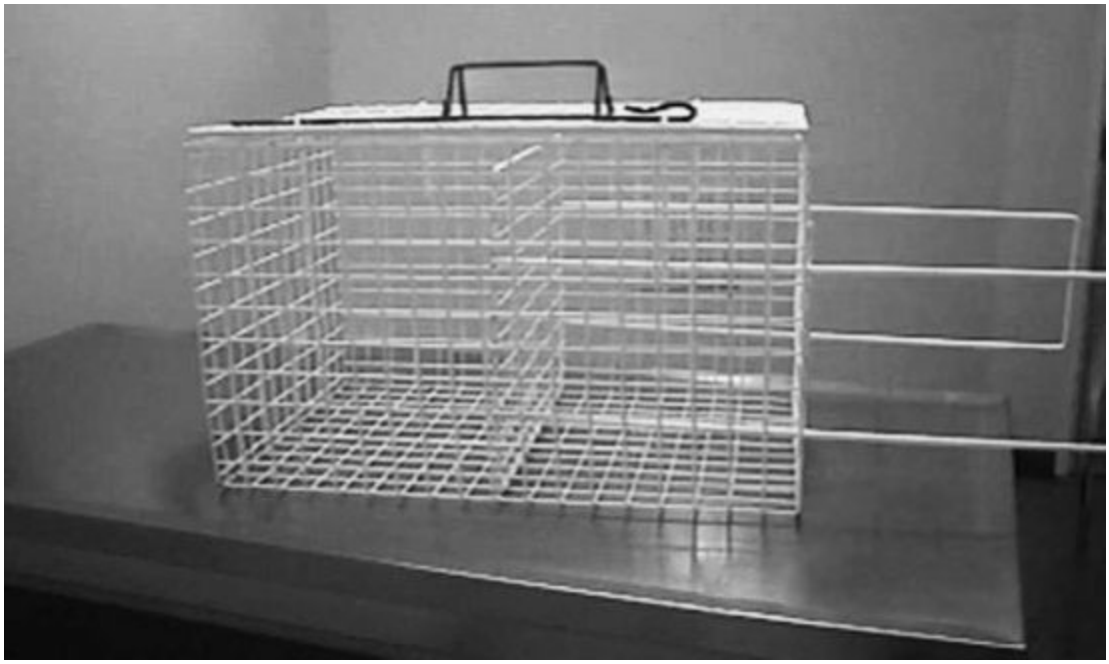
General considerations

If pharmacological restraint is required it should be given under veterinary supervision. Anaesthetics and analgesics suitable for the species and procedure should be used, which allow the animal to make a rapid return to consciousness. Animals should only be released when they have recovered sufficiently from the procedures to be able to cope with hazards in the environment. Since there may not be the opportunity to check on the animals post-operatively, surgical procedures must be performed to a particularly high standard, using strict aseptic techniques and good surgical and suturing methods. A wide range of smaller wildlife species can be successfully lightly anaesthetised for restraint using isoflurane, either alone or in combination with injectable agents. For larger species such as badgers, otters and foxes induction of anaesthetic is generally by injectable agents. These are administered through the mesh of the trapping cage, such as a crush cage (see Figure 15.2), or by means of a blowpipe or dart gun. Use of these is controlled under the UK Firearms Act (1968) and requires permits. Many commonly used injectable combinations include non-reversible agents with associated prolonged recovery times. This is generally undesirable in the field where there are unlikely to be facilities to maintain adequately warm temperatures; it is difficult for personnel to monitor the recovery of more than a few individuals and animals are prevented from resuming normal activities (for example, hunting for food or protecting

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young). Well-controlled anaesthesia is important for the operator: an animal suddenly recovering from anaesthesia is likely to bite and so present a risk of injury and transfer of zoonotic disease.

FIGURE 15.2 Crush cage for use with small wild animals.



Although fieldwork presents particular challenges, many of the procedural refinements used in the laboratory or surgery can be transferred. A folding table (such as of the type used for pasting wallpaper), covered in thick polythene sheeting and disposable paper covers, functions well as an operating table which can readily be disinfected between animals (see Figure 15.3). Some of the problems encountered in the trapping and subsequent anaesthesia of small wild animals are due to their very small size and high metabolic rate. Their high ratio of surface area to body weight is such that they lose heat rapidly and hypothermia must be prevented. All animals should therefore be placed on bedding, over gel heat pads or hot-water bottles, during anaesthesia and recovery. They must be placed in sheltered positions, provided with food, and their holding cages covered with blankets or bubble wrap during recovery. Death rates in traps can be particularly high for shrews, possibly because of their very high metabolic rates and thermal requirements. Small birds also have a higher basal metabolic rate (BMR) than larger ones: a hummingbird weighing 3.5 g has a BMR of around 6720 kJ/kg per day, whereas a swan weighing 9 kg has a BMR of around 200 kJ/kg per day. This high metabolic rate renders birds prone to hypoglycaemia even if starved for only a few hours. The heart is relatively large and beats very fast, and birds will readily develop acute cardiovascular failure if stressed. Their body temperature is often 41°C or more, which allows the rapid metabolism required, but which also renders birds prone to hypothermia. Body temperature is regulated by physiological and behavioural means which varies between species depending on their natural habitat. Birds will fluff up their feathers, take shelter and shiver when it is cold, but they have limited capacity to lose heat if it is warm. They do not possess sweat glands, and therefore heat loss can only be effected by panting or gular fluttering, which is the rapid movement of the thin floor of the mouth and upper area of the throat. This must all be taken into account when they are anaesthetised.

FIGURE 15.3 An operating table that can readily be disinfected between animals.

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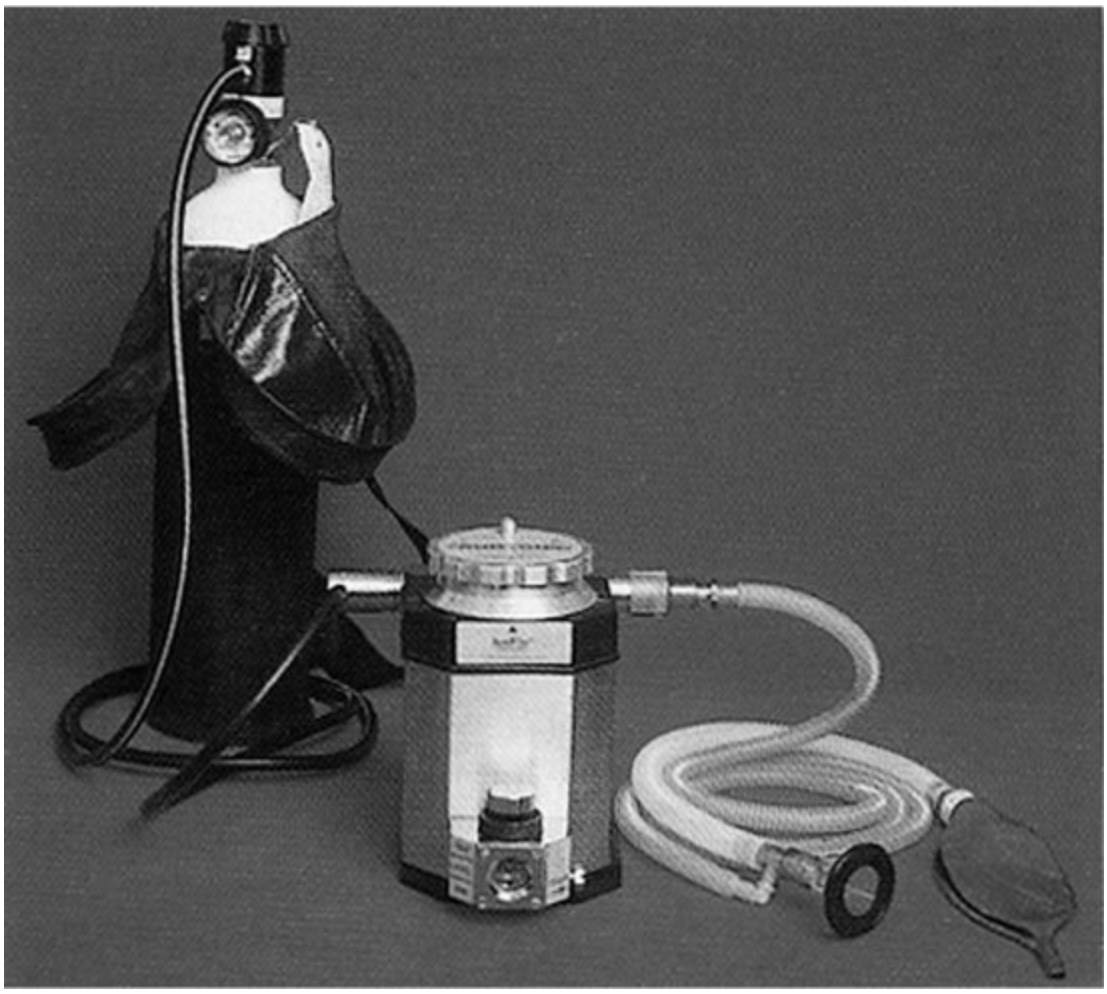


Inhalational anaesthesia

Apparatus is available for inhalational anaesthesia in the field that is light enough to be portable in a simple backpack[7]. An isoflurane vaporizer, which weighs approximately 7 kg, is attached to a portable oxygen cylinder (see Figure 15.4). It is important to note even with a temperature-compensated model, prolonged exposure to temperatures below 10°C (for example, overnight storage in a vehicle in winter) will adversely affect the calibration for some time even after air temperatures have risen. This is due to the slow rate at which the vaporizer is designed to expand and contract. This problem can be overcome in the field by protecting the vaporizer from protracted exposure to low temperatures using an insulated box, if necessary supplied with hot-water bottles. The vaporizer is connected using standard circuitry to a portable 230 litre medical oxygen cylinder (weight 2.9 kg). The cylinder can be refilled from a standard-sized canister using a recharging adaptor. The cylinder should be fitted with a regulator permitting flow rates of 1–15 litre/min.

FIGURE 15.4 A vaporizer, which weighs approximately 7 kg, is attached to a portable oxygen cylinder.

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The protocol used for induction depends on the size of the animal. Small mammals caught in metal box Longworth traps can be gently tipped with their bedding into a transparent polythene bag. This bag then serves as an induction chamber, with the neck of the bag being held closed around the anaesthetic gas tube (see Figure 15.5). Larger species caught in wire mesh traps, such as squirrels, rats and rabbits, can be anaesthetised as above, except the whole trap is placed inside a polythene bag. Larger animals such as badgers and foxes are better given short-acting reversible injectable anaesthetic for induction (Figure 15.6). All species are then transferred to a face mask or intubated for maintenance (see Figures 15.7 and 15.8). Since fieldwork is usually conducted outside there may be no need for a scavenging system to be included in the circuit. The main advantage of this inhalation system is its safety for the animal, despite their unknown health status at induction. In addition, the animals recover very quickly from isoflurane: most are fit for release within 20 min of the end of anaesthesia and can be returned to the wild with less disruption to them and to their population than using injectable methods alone.

FIGURE 15.5 Induction chamber, with the neck of the bag being held closed around the anaesthetic gas tube.

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FIGURE 15.6 Larger animals such as badgers and foxes are better given short-acting reversible injectable anaesthetic for induction.



FIGURE 15.7 All species are then transferred to a face mask or intubated for maintenance.

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FIGURE 15.8 Badger with an endotracheal tube.



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Drug doses and body size

When administering drugs to wild animals it is common to extrapolate doses from other species where the dose rate is known. However, smaller animals have a relatively higher basal metabolic rate and so the dose required is not dependent on the body weight (W, kg), but on the metabolic body weight, which can be obtained by $W^{0.75}$, and the energy group to which the animal belongs. Animals can be divided into five energy groups depending on the mean core temperature range (see Table 15.1)[8]. Hainsworth identified constants for each group, and when the metabolic body weight is multiplied by the Hainsworth constant the product can be used to set up ratios for scaling pharmacokinetic or physiological parameters from one species to another. This process is known as allometric scaling.

Table 15.1 Hainsworth's energy groups.

Group	Constant (K)	Mean core temperature (°C)
A Passerine bird	129	42
B Non-passerine bird	78	40
C Placental mammal	70	37
D Marsupial mammal	49	35
E Reptile	10	37*
*Requires body temperature to be held artificially at 37°C.		

To extrapolate dose rates or frequencies from one species to another the animal's specific minimum energy cost (SMEC) must be calculated. This is given by:

$$\frac{K(W^{0.75})}{W} = K(W^{-0.25})$$

Then, calculate the test dose or frequency by extrapolating from a known dose rate or frequency:

$$\frac{\text{Control dose or frequency}}{\text{Control SMEC}} = \frac{\text{Test dose or frequency}}{\text{Test SMEC}}$$

For example, if the dose of a drug for a 10 kg dog is 10 mg/kg twice daily, the dose D and frequency F for a 20 g mouse can be calculated by:

$$\frac{10}{70 \times (10^{-0.25})} = \frac{D}{70 \times (0.02^{-0.25})} \quad \text{therefore} \quad D = 47.3 \text{ mg/kg}$$

$$\frac{2}{70 \times (10^{-0.25})} = \frac{F}{70 \times (0.02^{-0.25})} \quad \text{therefore} \quad F = 9.4$$

So, a mouse needs 47.3 mg/kg of the drug 9.4 times daily, or every 2.5 h.

These formulae can be used to calculate reasonable, effective dose regimes for any species of warm-blooded animal, ranging from the smallest birds to large ungulates.

Drug doses for wild animals

The following regimes may be used as guidelines for injectable anaesthesia of some particular species of wild animals[9], pigeons and doves[10] and European starling[11].

Rats, mice, voles, rabbits

The doses described in Chapter 9 may be used, remembering that the health and condition of the animal may not be as robust as an animal in the laboratory.

Squirrel

Medetomidine 250 µg/kg plus ketamine 60 mg/kg administered together intramuscularly (i.m.). Reverse with atipamezole at 1 mg/kg subcutaneously.

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Hare

Give medetomidine 0.25 mg/kg i.m. Adjust according to response. Reverse with atipamezole and adjust according to response. Try a low dose and give more if necessary after 3–5 min. Ketamine may be used if medetomidine does not prove successful.

Hedgehog

Ketamine at 20 mg/kg produces sedation with recovery in 30 min.

Badger

Ketamine at 15–20 mg/kg i.m. produces immobilisation, and at 30 mg/kg produces deep tranquillisation. This lasts for about 45 min but will take much longer to work if the environmental temperature is low. For general anaesthesia 20 μg/kg medetomidine with 4 mg/kg ketamine plus 0.8 mg/kg butorphanol is an excellent combination. Alternatively, use 2.0 mg/kg xylazine with 7.0 mg/kg ketamine i.m. or 70–80 μg/kg medetomidine with 3.5–4 mg/kg ketamine i.m. Reverse with 350–400 μg/kg atipamezole i.m.

Otter

Medetomidine 40 μg/kg plus ketamine 4 mg/kg administered via a blow pipe to produce heavy sedation, then 30 μg/kg medetomidine plus 3 mg/kg ketamine given i.m. will produce 40 min of anaesthesia and can be reversed with 350 μg/kg atipamezole.

Birds

Ketamine combinations: for wildfowl, 15–50 mg/kg will give light surgical anaesthesia. It may be given in incremental doses of 5 mg/kg at 5 min intervals and can be used to maintain light anaesthesia/sedation for several hours. For small birds mix 0.1 ml of ketamine (100 mg/ml) in 0.9 ml of sterile water. Give 1 mg (0.1 ml) per bird i.m. This will produce sedation in 3–4 min and recovery in about 20 min. At 2 mg/bird there is light surgical anaesthesia for 5–12 min and recovery in 30 min. At 3 mg/bird there is 5–20 min of anaesthesia and recovery in 60 min. Ketamine may be used alone for induction prior to using isoflurane for longer procedures, but is also used in combination with other drugs. For deeper anaesthesia in surgical procedures where some relaxation of the muscles is required, ketamine at 20 mg/kg i.m. may be combined with midazolam (4 mg/kg i.m.) or diazepam (1.5 mg/kg i.m.).

Identification of Wild Animals

The ringing, tagging or marking of an animal for the primary purpose of identification is not regulated under ASPA if it causes only momentary distress and no lasting harm. However, best practice must be followed – as prescribed, for example, by the British Trust for Ornithology – and account taken of the ways in which identification techniques may compromise an animal's ability to function. Individual animals may be identified by their unique features, or by methods as used in other laboratory species. A variety of attachment devices are used with telemetric and GPS devices. These should be as light as possible and less that 5% of the animal's own body weight. Collars or harnesses should be prepared to allow for potentially wide variation in body size within the population and fitted to allow enough room for growth.

Leg rings are the most common method for birds. Tags in the ear or wing can be used in some species but care must be taken in use of colour so that they do not affect camouflage, and they should be positioned so they do not get caught on vegetation leading to tissue damage, especially in species that burrow[12]. Fur clipping can be used as a temporary marking method or tattooing (under general anaesthesia) as a permanent method. Microchips are also suitable, as is use of individual identification based on natural markings in some species. Methods that involve physical alteration of the animal such as ear notching or toe clipping are not recommended, since they can affect the animal's behaviour or ability to survive once released back into the wild.

Release of Animals Back to the Wild

Animals that have been taken into captivity and subjected to scientific procedures may need to be released back into the wild at

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the end of or during the procedures. The release of animals under these circumstances, however mild the procedures, requires the prior consent of the Home Office. ASPA requires that animals on which regulated procedures have been performed are killed at the conclusion of the series of regulated procedures, unless a veterinary surgeon or other competent person determines that they are not suffering or likely to suffer as a result of the procedures. In addition, if the animal is to be released at a place which is not an establishment with a Section 2C licence, a veterinary surgeon may need to verify that the animal will not suffer if it is released in that place. However, such work may be undertaken at remote sites (e.g. nature reserves), where it is not practicable for a veterinary surgeon to be in constant attendance. In such situations it may be necessary to rely on observance, by suitably trained and qualified persons, of a written protocol, which is not expected to cause lasting harm if followed correctly. The veterinary surgeon may then be assured that an animal will be fit, if the protocol is carefully followed by these trained and competent individuals. As part of conservation projects sometimes animals that have been bred in captivity are released into the wild and these also need to be assessed for fitness before release[13].

If animals are to be released to the wild in the course of regulated procedures – for example in studies where individuals are captured, measurements are made, then the animals are released and recaptured at intervals – additional safeguards must be put in place. If regulated procedures are applied to animals taken from the wild the holder of the licence authorising the work must ensure that the animals are captured by a competent person using a method which does not cause the animal avoidable pain, suffering, distress or lasting harm. The licence holder is also responsible for ensuring that if an animal taken from the wild is found to be injured or in poor health it is not subjected to a regulated procedure unless it has been examined by a veterinary surgeon or other competent person and action has been taken to minimise the suffering of the animal, unless an exception to this has been agreed in the conditions of the licence.

In addition to ensuring that the requirements of other relevant legislation have been met it is equally important to ensure the well-being of animals released into the wild after capture, even if they have been used for purposes not regulated under ASPA. In the case of research on farms or other partially protected sites it may be possible to exercise a degree of supervision after their release. Where possible there should be appropriate monitoring of the animal for unexpected adverse effects. The animals should not find themselves at a biological disadvantage as a result of the procedures performed or of its captivity. Regard should also be had to the welfare of other individuals (e.g. the offspring of those captured). The following considerations should apply:

- animals should not be released in such a way as unreasonably to expose them to risk (e.g. of predation, thirst or hunger),
- animals should be released at a suitable site, normally within their home range,
- the timing of the release should take account of the animal's normal daily and seasonal activity patterns and other relevant factors,
- where animals have been injured or found to be injured or unwell, account must be taken of relevant veterinary advice and welfare considerations,
- the duration of capture should be the minimum consistent with both the object of the experiment and humane release,
- consideration should be given to the consequences of the release of animals into the food chain.

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Chapter 16

Aquatic Species

Introduction

Aquatic species are increasingly used in scientific procedures, for fundamental studies such as developmental biology and applied research. A total of 489 047 fish were used in scientific procedures in the UK in 2010, the majority in fundamental studies, but significant numbers in applied studies including environmental studies such as ecotoxicology. Fish therefore represent the second largest group of animals used in research, being second only to mice. Over 13% of the 3 642 517 animals used were fish[1]. In addition, 6319 amphibia were used, mainly in fundamental research (0.4% of animals used). These animals therefore represent a significant proportion of those used in scientific procedures and researchers increasingly need to become familiar with the biology and husbandry of aquatic species.

Fish

Fish are a very diverse group of animals, naturally inhabiting a vast range of habitats. It has been estimated that fish account for almost half of known vertebrates[2], with about 28 000 species. Fish can be divided into bony fish (Osteichthyes or teleost fish, 96%), cartilaginous fish such as sharks and rays (Chondrichthyes, less than 4%) and jawless fish such as lampreys (Agnatha or cyclostomes, less than 1%).

Fish are able to adapt to environmental pressures to a greater extent than mammals and birds, some species even being able to change sex according to conditions. A detailed discussion of the huge range of species is beyond the scope of this book. A brief introduction is provided for two commonly used species, zebrafish (*Danio rerio*) and rainbow trout (*Onchorhynchus mykiss*). More information can be found in the *UFAW Handbook on the Care and Management of Laboratory and Other Research Animals*, 8th edn2 and the guidelines published by the Canadian Council on Animal Care[3].

Although there is a lack of published research into the husbandry needs of many species of fish, fish have been kept in captivity in laboratory and commercial settings for many years so satisfactory husbandry practices have been developed. The study of fish welfare is an expanding field, and researchers are advised to consult the latest research for up-to-date information on these species.

Sources of fish

Commonly used fish species are generally obtained from commercial stocks, but there is likely to be considerable variation within and between stocks, even in a genetically homogenous population, due to the plasticity seen in fish. Where possible, fish should be sourced from a reputable supplier with a high health status and known genetic makeup.

General biology

Most fish are ectothermic, and biological processes including growth, healing and reproduction are temperature-dependent. Ectotherms do not have to maintain body temperature and therefore can survive on much less food than mammals and birds. Some are very adaptable, being able to survive in salt and fresh water, whereas others have stricter environmental requirements.

Reproduction varies with the species, some fish producing large quantities of eggs and providing no protection for the young, others being live bearers, producing smaller numbers of eggs which are fertilized and hatched within the female.

Fish exhibit a wide range of complex behaviour patterns and have different environmental requirements. These vary with species. Some species have to be housed individually, whereas others are best kept in groups. An appropriate stocking density is

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important to minimize territoriality and aggression, but reducing the stocking density does not automatically improve welfare: other factors are involved, including water quality and resource availability2. Specialised texts should be consulted for more information on housing of individual species.

Observation of behaviour should be undertaken other than at times of disturbance (feeding, cleaning), since the fish will not be exhibiting normal behaviour at these times. Many fish species, including salmonids, are territorial, and may show aggression if competing for resources. This can be prevented by avoiding defensible resources and visual cues, which can be used to define territories. Providing a covered area can reduce predator-avoidance behaviour (rapid swimming from group to group or crowding together into areas of shade)2.

Biological data

Variation within and between species of fish is greater than for terrestrial animals, and normal values for haematology and biochemistry are generally not useful2. Growth depends on numerous factors, and growth charts produced by commercial suppliers may not be appropriate for laboratory situations. Therefore records of biological parameters for the fish in the particular system being used should be kept, to enable meaningful conclusions to be drawn from analysis of any subsequent data.

Husbandry

The major requirement for husbandry of aquatic species is keeping the water clean and aerated. Otherwise animals are exposed to toxins from their own excreta. To achieve this they should be either kept in static water systems at low stocking density and the water changed regularly, or kept in circulating systems with biological filters to remove toxins.

Rooms for fish need a high-quality water source, a sink, a floor drain and a power supply, and must be able to support the weight of the tanks. All fittings need to be able to withstand high humidity and exposure to water. If circulating systems are used, back-up pumps are advised in case of equipment failure.

Tanks can be made from glass or plastic (latter may become scratched). Tanks should be kept on a dark surface away from light, and tubing carrying fresh and waste water should be non-transparent, to minimise algal growth.

For all fish some treatment of the water is required. As a minimum, tap water should be passed through a charcoal filter to remove organic compounds and chlorine. If water has been treated by reverse osmosis to remove all salts, then salts need to be added to produce an appropriate pH and conductivity. Aeration is always required for reverse-osmosed water and after charcoal filtration.

Non-filtering systems do not require the establishment and servicing of biological filters, but need considerable space due to the low stocking density and high maintenance requirements. Changing a proportion of the water daily is needed to keep nitrite levels down.

In recirculating systems water is subject to mechanical filtration or manual cleaning with vacuum pumps to remove debris, then passed through charcoal and biological filters to remove toxins. Finally it is aerated before being reused. Ultraviolet sterilisation can also be used to reduce microbial growth. When using a large-scale recirculating system water from many tanks can be cleaned by a single unit. The disadvantage is that diseases can be spread between tanks, although ultraviolet sterilisation after filtration may help. Alternatively several smaller units with individual filters can be used to prevent spread of disease but these require more maintenance. Fish may be harmed if the water stops running for a significant period. If the filters are not working properly the pH can rise above 8. This prevents growth of denitrifying bacteria and leads to build up of toxic material. Water may also need additional aeration: denitrifying bacteria need oxygen so filters work better in aerated water.

Biological filters usually consist of boxes of material with a large surface area, either within the tank or outside the aquarium with water pumped through. Filters act as mechanical filters, but also provide a large surface area for colonisation with denitrifying bacteria, which degrade toxic ammonium compounds to nitrate, which is less toxic. Care must be taken when using chemicals in the system since disruption of the bacterial filter can result in a rapid deterioration in water quality.

Biological filters need a start-up phase, during which denitrifying bacteria colonise the filter mats, which can take several weeks. This can be speeded up by seeding the filter with water from an established aquarium, or with proprietary preparations. Water should be checked daily for ammonium compounds and nitrate, and water exchanged if these rise. New fish should be introduced gradually over a period of 1–2 months to allow the filters to adapt.

Regular cleaning of tanks is required in all systems. Algae grow on the walls of the tanks and should be removed regularly. Some facilities use snails from disease-free sources in tanks to keep algae in check, although fish may eat these. Different siphon tubes should be used for each tank and sterilised between tanks[4], and tanks should be cleaned thoroughly between batches of fish.

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Cage washers may not be sufficient to thoroughly disinfect tanks and they may need to be manually scrubbed. Tanks can then be autoclaved or treated with disinfectant, for example hydrogen peroxide[5].

Water quality

Water quality affects the health of fish, although there is limited data on the effect of poor water quality on anything other than survival or growth. Effects on behaviour or other indicators of welfare2 are largely unknown. Table 16.1[2,5,6] lists water-quality standards that have been found to be suitable for growth and production in aquatic animals.

Table 16.1 Water-quality requirements for aquatic species2,5,6.

Parameter	Zebrafish	Rainbow trout	*Xenopus*
Temperature (°C)	18–28 (25–28 optimal)	12–21	18–20
Dissolved oxygen (mg/l)	>6	>5	>5
Carbon dioxide (mg/l)	<5	<2	<5
Ammonia (mg/l)	<0.02	<0.0125	<0.2
Nitrite (mg/l)	<1		
Nitrate (mg/l)	<50		<0.3
Chlorine (mg/l)	0		<3.8
pH	6–8	6.5–8.5	6.5–8.5
Alkalinity (mg/l)	50–150	10–400	150–250
Hardness (mg/l)	80–300		
Manganese (mg/l)		<0.01	
Zinc (mg/l)		<0.05	0
Copper (mg/l)		<0.006 (soft water), <0.3 (hard water)	0
Conductivity (μS)	300–1500		400–1200
Salinity (g/l)	0.5–1		0.6

The speed with which these parameters may change depends on numerous factors including the ratio of fish biomass to water volume and the capacity of the filtration system. Therefore no single monitoring regime is appropriate for all systems[2]. As a minimum, in flow-through systems, measure temperature and dissolved oxygen at least once daily, with pH and ammonia checked at least weekly. In static systems measure temperature and dissolved oxygen daily, and other values depending on the frequency and amount of water exchange.

Feeding

Fish need relatively little food compared with mammals. Short periods of starvation appear not to be detrimental whereas overfeeding has many adverse effects. Different species have different requirements, and specialised texts should be consulted for detailed information. In general, first feeding, when the recently hatched fish have used their yolk sacs and are starting to feed independently, can be a critical period, and care must be taken to ensure appropriate foodstuffs are offered at this time.

Handling and Techniques

Handling is stressful for fishes, although they can become conditioned to handling to some extent. Even routine handling procedures may cause injury or even death if carried out incorrectly. Fishes should be handled only when necessary, and the number of handling episodes should be minimized. Appropriate handling equipment, such as a bucket or knot-free nets, should be used to minimize damage. Fish should be handled very gently, and kept moist at all times. They should always be placed on soft, non-abrasive, wet surfaces2.

Digestion requires energy and increases oxygen demand, so fish will defecate and vomit in response to stress to conserve energy2,3. Fishes should therefore be fasted prior to any procedure, including handling or transport. For small fish (<400 g), 24 h of fasting is sufficient, with 48 h for larger fish2. Fasting also reduces ammonia output and water contamination. High-quality water for procedures and recovery should be provided so that any gut emptying that occurs does not cause welfare problems.

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The skin and mucous membranes of fish act as barriers to osmotic stress and infectious agents, and damage can lead to significant morbidity. The epithelium is thin, with a covering of mucus. In some species the skin is delicate and easily damaged by handling, especially prolonged restraint. Handling can also cause myopathy. Anaesthetics and sedatives are frequently used to prevent external damage during procedures.

Procedures should be conducted quickly and competently to minimise stress. Longer procedures may be best done under sedation unless the fish have been conditioned to handling. Recovery from the stress of procedures may take from a few hours to several days. Where fish are to be handled repeatedly, a suitable period of recovery is required between procedures. Some water additives are available to help maintain the integrity of the skin and aid recovery after procedures.

Many fishes are sensitive to visual stimuli, especially light, so handling in a dimly lit area may help lessen handling stress.

In general, fishes should not be kept in air continuously for more than 30 s. The length of time fishes are held out of water should be minimized, and should not exceed 30 s for most species. Even brief periods out of water can cause damage to gills, so these need to be kept moist3.

If fish are to be transported, small groups of fish can be placed in sealed plastic bags or boxes containing one-third tank water and filled with oxygen. During transport temperature should be maintained as far as possible, and on arrival fish should be acclimatised to the new environment: the water conditions should be as similar as possible, and the temperature in the transport water and receiving tank allowed to equilibrate before the fish are released into the new container. Fish should be closely monitored after transport for signs of ill health.

Identification

For some fish it may be possible to identify them from markings, avoiding the need for invasive marking techniques. Anaesthesia or sedation may be required for tagging or marking. No single tag or marking method is suitable for all situations.

Tagging involves stress and injury, from both handling and the wound caused by application of the tag3. Tags should be chosen which allow normal behaviour and minimise risk of entanglement. The weight, shape and size of the device must be considered. Brightly coloured tags may compromise an animal's camouflage. Various types of internal and external tag, including small tags and passive integrated transponder (PIT) tags for subcutaneous and abdominal implantation, are available. Alternatively, small quantities of coloured compounds can be injected subcutaneously to mark the fish.

Marking techniques that cause significant tissue injury, such as freeze branding, tattooing or clipping important fins, should only be used if alternative methods are not suitable. Removal of even a small part of a fin can be detrimental to the ability of the fish to swim2.

Anaesthesia

Fish should be starved for 12–24 h before anaesthesia to prevent regurgitation. Anaesthesia can be achieved by immersion in a solution of appropriate anaesthetic. Tricaine methane sulphonate or MS222 (Pharmaq MS222, Pharmaq) is licensed in the UK for this purpose. MS222 produces dose-dependent sedation and anaesthesia, depending on the species and with individual variation. The level of anaesthesia depends on water temperature, water hardness, salinity, oxygen concentration and duration of immersion. Smaller fish tend to be more sensitive. Water for anaesthesia should be taken from the holding tank. The required amount of MS222 should be weighed and dissolved in a small quantity of water, then this added to the anaesthetic bath, in to which the fish are then placed. The water should be aerated to maintain oxygen levels and remove carbon dioxide. MS222 can be acidic, and water pH should be measured before and after adding the agent. If there is a reduction in pH of more than 1 pH point buffering may be necessary, using sodium hydrogen carbonate, to raise the pH to 7–7.5. This is less likely to be necessary with hard water at concentrations used to induce anaesthesia[7].

Since fish vary in their sensitivity, a low initial anaesthetic concentration should be chosen and tested on a small number of fish, and more anaesthetic added if required. Initially there will be increased opercular movement, followed by a reduction in opercular movement, increasing ataxia and loss of balance before immobility. It may take 10 min for anaesthesia to be induced. Depth of anaesthesia can be judged by respiratory rate and (lack of) response to pinching the tail base. If anaesthesia is to be maintained a lower concentration of anaesthetic can be trickled over the gills (50–100 mg/l).

Following the procedure the fish should be placed in clean water and will return to consciousness within 1–30 min.

Table 16.2 gives suggested induction doses for MS222 in different species. Anaesthesia can also be induced and maintained with isoflurane, or with a mixture of MS222 and isoflurane. Isoflurane can be added to the water at 0.4–0.75 ml/l for induction, reducing to 0.25–0.4 ml/l for maintenance. Other anaesthetics used in fish include benzocaine and clove oil[8].

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Table 16.2 Induction of anaesthesia with MS222

Species	Sedation (mg/l)	Light anaesthesia (mg/l)	Deep anaesthesia (mg/l)
Zebrafish	30–50	50–100	100–200
Rainbow trout	10–30	30–80	80–180

Administration of compounds

Immersion

The most common route for administration is via the gills. Fish gills have a large surface area and a thin epithelium for gas transfer, which also allows for absorption of other molecules. Compounds for administration by this route are dissolved in a quantity of tank water, usually in a separate smaller vessel. Fish must be observed for signs of ill health during the period of exposure and the water aerated or changed as appropriate to maintain water quality.

Oral

Fishes may be force-fed liquids and semi-solid solutions using flexible rubber tubing and a syringe. Anaesthesia may be necessary to prevent struggling and vomiting. In some species a rigid speculum may be used to aid passage of the tube. Regurgitation may occur, so fish should be carefully observed to ensure the administered agent is retained. In general, no more than 1% body weight should be administered orally in a single dose3.

Injection

Common routes for injection in fish are intravascular, intraperitoneal and intramuscular. Care should be taken during injection to introduce the needle in spaces between the scales. Intramuscular injections may be made into the large dorsal epaxial and abdominal muscles, taking care to avoid the lateral line and ventral blood vessels. Intraperitoneal injections should avoid penetrating abdominal viscera.

Implants and slow release devices can be surgically implanted in the peritoneal cavity or implanted with a trocar into muscle.

Collection of samples

Samples of blood or tissues may be required for study. Some samples may be taken from anaesthetised fish which are then allowed to recover, but often with small fish samples are taken post mortem. Fish deteriorate very rapidly after death, and samples must be taken often within a few minutes to be of value2.

Blood

In general, blood sample volume up to 1 ml/kg of body weight is recommended. The fish must be permitted to recover their haematocrit prior to subsequent blood collection. Haematocrit recovery times are temperature-dependent and highly variable between species.

Methods for blood collection are listed below[2,9].

- Tail ablation after euthanasia: fish that are too small to bleed with needle and syringe may need to be sampled after euthanasia. The caudal peduncle is severed with a scalpel and blood collected with a capillary tube.
- Caudal venous puncture: the caudal vessels lie under the spine. The fish is placed on its side and the needle is inserted in the ventral midline just behind the anal fin angled slightly forwards. Alternatively, a lateral approach can be used, inserting the needle between the scales just below the lateral line in the middle portion of the tail base. The needle is inserted slowly upwards towards the vertebral column until the bone is reached, then the needle withdrawn slightly and the sample taken.
- Cardiac puncture: this is a tricky technique and should only be carried out after extensive training and practise on cadavers. The needle is placed perpendicular to the skin and inserted slightly below the gill cover.

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Surgery

The mucus and skin of fish contain antibacterial agents, and removal of mucus and disruption of scales during surgical preparation may render the area susceptible to infection, so the surgical scrub procedures used in mammalian surgery are not recommended. Gentle removal of grossly visible dirt and debris may be sufficient, followed by cleaning with dilute aqueous povidone/iodine solutions. Quaternary ammonium compounds may be irritant and should be avoided. If it is necessary to remove scales they should be removed individually by pulling in a posterior direction to minimize damage. Scale removal results in an injury which extends into the vascular tissue. The use of sterile occlusive drapes can help maintain a sterile field. Sterilised cling film can be used for this purpose: this can be wrapped round the fish and the incision can be made through it. Sterile instruments should be used.

The incision should avoid the lateral line and should follow the longitudinal axis of the fish. The epidermis and peritoneal layers are easily torn, but overall fish skin is tough. Abdominal incisions may be made on the ventral midline or lateral to this region. Ventral midline incisions avoid muscle tissue, but the incision may come in contact with the substrate in the tank, causing damage to the incision site. Lateral approaches avoid this problem, but invade muscle and may result in accidental puncture of underlying organs.

When closing incisions, since the skin layers are all closely adherent to each other, a single layer of sutures through all layers is sufficient in most cases. Suture material should be monofilament, inert and placed with an atraumatic needle. Internal layers and organs can be closed using synthetic absorbable materials. Tissue adhesives may not be suitable, as they only bond dry material so may not hold in aquatic environments.

In general, fish skin heals faster than mammalian skin; however, fibrous proliferation can be slow and varies with temperature. Factors that affect wound healing include water quality, presence of tank mates, nutritional plane before surgery and speed of return to normal feeding, hormonal status, integrity of mucus layers and skin, and the presence or absence of opportunistic pathogens.

Post-operative care and analgesia

Little is known about the effect of analgesic drugs on fishes. However, post-operative analgesia should be given where appropriate as suitable analgesic agents become available. Fishes do appear to produce opioid substances in response to pain and fear, similar to higher animals[10,11,12,13], and respond to analgesics similarly to mammals.

There may be prolonged metabolic effects following anaesthesia and surgery, therefore careful monitoring and care for several hours in the post-anaesthetic period are needed. A quiet, well-aerated, possibly darkened tank with clean water where the fish can be easily observed is recommended for recovery, where there is minimal environmental stimulation and a consistent temperature. Post-anaesthetic complications may include wound dehiscence and infection, osmotic imbalances related to surgical incisions, and anorexia.

Care is needed when returning the fish to the pre-surgery tank. The benefits of companion fish (social interaction, schooling fish and feeding activity stimulation) have to be weighed against the disadvantages (predation, competition for feed and biting of sutures). Shelters for recovering fish and water-conditioning agents may help speed recovery3.

Health and disease

Fish used for research should be free of diseases, since these can affect results. There should be procedures in place to limit the introduction of disease into the facility, and a programme for the detection and management of disease conditions and water-quality problems. Disease-control measures, such as quarantine, immunisation and prophylactic treatments, and a system of regular health monitoring should be in place3. Handling should be minimal to minimise stress. Many diseases in fish can be prevented through good husbandry and biosecurity measures. New fish are one of the largest risks for introduction of disease, so new stock should be sourced from colonies free from infectious diseases, and quarantined for a suitable period (3 weeks or more2) on arrival. Sick fish should be removed from the colony immediately. Mixing different groups of fish should be avoided. Water is another potential source of infection, so incoming water should be treated if possible to minimise the risk of introducing disease. Ideally separate tanks should receive separate supplies of water: systems which circulate water between different tanks facilitate the spread of disease. An ‘all-in all-out’ policy with disinfection of the system between batches of fish is recommended2. Shared equipment such as nets should be properly disinfected between tanks.

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Recognition of pain and distress

It is only recently that studies have been conducted into the cognitive capacity of fish. Recent studies have identified nociceptors in fish, and fishes respond to noxious stimuli with altered behavioural, physiological and hormonal parameters. The responses seen are similar to those seen in more highly evolved vertebrates[14]. There has been debate whether these responses demonstrate that fish have a capacity for fear and suffering[14], or are simply responding passively to stimuli[15,16]. However, there is increasing evidence that fish have the capacity for long-term suffering[17]. Either way, there is conclusive evidence of a stress response in fish that can be detrimental to physiological processes, irrespective of the cognitive abilities of the animal. Therefore it is essential to minimise stress when managing fish in the laboratory.

The recognition and evaluation of pain and distress in fishes is not easy. Many fish species are prey animals and do not exhibit overt signs of injury or pain. Time should be taken to observe and record the normal behaviour and appearance of the fish in the particular setting, to facilitate identification of any abnormalities2. Reluctance to eat, unusual behaviour, discoloration of the integument and lesions are signs of possible developing health problems3. Signs of ill health also include reddening around the mouth and vent, swimming close to the surface or erratically, inability to maintain posture, swollen abdomen, lethargy, darkening, blue-grey slime or white patches on body, loss of condition, or a cloudy lens in the eye. One approach is to draw up a checklist of anticipated and potential clinical signs for each study, including 'population' clinical signs (such as feed intake) and 'individual' clinical signs (such as skin lesions). End points can then be defined for each study (see Chapter 4 for more information on end points).

In the long term, the effects of stress can include loss of appetite, inhibition of growth, impaired reproductive success and impaired immune response.

Euthanasia

EU Directive 2010/63 permits euthanasia of fish by overdose of anaesthetic, electrical stunning or concussion, although electrical stunning is not permitted under Schedule 1 to the Animals (Scientific Procedures) Act 1986. Overdose of anaesthetic is often achieved by use of a buffered solution of MS222 (tricaine methane sulphonate) at a concentration of at least 250 mg/l. Death usually occurs within a minute, but animals should be kept in the solution until death is certain, at least 10 min after opercular movements cease. Fish may also be given an overdose of pentobarbitone (60–100 mg/kg) by intravascular or intraperitoneal injection.

Where feasible, the euthanasia of fishes using physical methods should consist of a two-step process, with initial anaesthesia followed by a physical method to cause brain death, except when animals are in distress and immediate euthanasia is required. A stunning blow is administered to the head, followed by pithing or cervical dislocation. Many species of fish continue to have brain activity when there is cerebral and systemic hypoxia; therefore physical euthanasia techniques such as decapitation alone should be avoided. It is recommended that the brain is destroyed in fishes that have been euthanised using a physical technique.

Various forms of electrocution have been used commercially; however, these can cause spinal fractures and muscle damage.

Zebrafish

Zebrafish, *Danio rerio*, are teleost fish of the Cyprinid family. They are widely used in studies of developmental genetics, neurophysiology, biomedicine and toxicology. Their genome consists of 1.7 gigabases, which is just over half of that of mammals, divided into 25 chromosomes5. Many wild-type and genetically modified lines are available (see http://zfin.org for details)[18].

Zebrafish are found naturally in slow-flowing shallow turbid pools in south and south-east Asia, often in unshaded locations[2,19]. They appear to inhabit floodplains rather than rivers. They inhabit the whole water column, including open water and among aquatic vegetation.

Zebrafish are small (up to 40 mm long) and robust, and large numbers can be kept in the laboratory. Females tend to be larger than males.

The natural history of the zebrafish is not well understood, thus husbandry protocols are not well developed and will vary between laboratories. Zebrafish tolerate a range of conditions, but production will be optimized within a set of parameters19. The optimum ranges have not been established and the recommend parameters are based on practices that have been found to be successful[2].

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Sources of fish

Eggs or larvae should be acquired from clean sources, but it is wise to keep new stock in quarantine for 2–3 weeks, checking daily for signs of disease, before moving into the main facility[5]. Adult fish should not be allowed to leave the quarantine facility: eggs obtained in the quarantine room should be bleached4 before leaving the quarantine room to be reared in main facility.

Behaviour

Zebrafish are a shoaling species, and establish dominance hierarchies that do not seem to be related to sex or size. Zebrafish can be aggressive at low densities, and may chase or bite subordinate fish19. As density increases aggression may reduce, but stress occurs in crowded conditions so they are best kept at intermediate densities. Aggression can be reduced by adding complexity to the environment – for example, plastic grass – so the fish can hide from each other, and fish are less aggressive in a covered habitat. Once dominance is established, aggression reduces. When mating male zebrafish may show territorial defence and active pursuit of females. Zebrafish release a pheromone when there is damage to the epidermis, which causes alarm in other fish. When alarmed there is an increase in shoaling behaviour, and either agitated swimming or freezing on the substratum, reduced appetite and increased aggression. There are differences in behaviour between strains of zebrafish.

Husbandry

Zebrafish are commonly kept in commercially available recirculating systems where several tanks are held on a rack and supplied by a single filtration column. The stocking density depends on the efficacy of filters, the age of the fish and the amount of feeding. In recirculating systems families of adult fish can be kept in serial tanks at five adult fish per litre.

In non-filtering systems fish must be held at a low density. Adult zebrafish can be maintained for up to 10 days without feeding or water exchange at a low density (two fish per litre). For longer periods regular water exchange and feeding is needed. Juvenile fish can be held in static tanks at at 60 per 2.5 litres until they are large enough not to pass through filters (2–3 months).

Zebrafish are surface-living, so tank height of 25 cm is sufficient provided there is adequate surface area. A well-fitting clear plastic lid is needed with a feeding hole smaller than 1 cm; otherwise fish can readily escape.

Zebrafish need temperatures between 25 and 28°C. The room temperature can be set higher to prevent condensation and growth of mould on tanks. Higher temperatures reduce oxygen levels in water, so if heaters fail and water drops to room temperature it is not dangerous. If temperature falls outside 25–31°C they may not breed or may show abnormal development4.

Zebrafish usually have 14 h light/10 h dark cycle. Fish do well in dim light, and bright light encourages algal growth. Red light is often used so researchers can see fish during the dark phase without encouraging algal growth.

Feeding

The exact feeding requirements of zebrafish are unknown19. Zebrafish appear to be omnivorous, their natural diet consisting mainly of zooplankton and insects. Adult fish not being bred require little feed and can be fed twice weekly. For breeding, fish require rich feeding. Typically these animals are fed dry food twice daily, with live food such as *Artemia* or bloodworms being added twice weekly or more, as dry food may not be sufficient5. Care must be taken not to overfeed the fish as degenerating surplus food impacts on water quality, and tanks need cleaning daily to remove excess food and faeces.

Breeding

Although in the wild zebrafish appear to breed annually, in the laboratory they can breed all year round. Zebrafish mate and spawn at dawn, and females spawn in response to male pheromones. To breed, pairs of fish can be set up in mating tanks in the afternoon or evening, with plastic grass to reduce aggression. Lids are essential as zebrafish can jump. Most fish will spawn the next morning, although some will only lay the second day. The male will chase and nudge the female, and may lead her to a spawning site. He will remain close to her and release sperm as she lays her eggs. Females can spawn every 2–3 days, and a single clutch typically contains 100–200 eggs2, but can contain as many as 700 eggs19. In the laboratory they spawn when the light is turned on in the morning, and the light cycle can be manipulated to produce eggs when required for study5. There is no parental protection2, and indeed eggs may be eaten by their parents. Eggs can be protected by using glass marbles at the bottom of the tank, or by using a mating container, which has a removable inner container with a perforated base.

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Eggs should be collected before noon the day they are laid. Unfertilised eggs promote growth of bacteria and mould, so eggs need to be removed rapidly into petri dishes containing suitable medium, and dead eggs removed regularly. Eggs may be bleached prior to raising to reduce the risk of disease4.

In vitro fertilisation can be used to obtain large numbers of synchronously developing embryos. Gametes are expressed from breeding adults by squeezing under anaesthesia4.

Hatching occurs between 48 and 72 h post-fertilization at 28.5°C2,19. Development is very rapid, with organs beginning to be identifiable from 36 h. After hatching, larvae attach to hard surfaces, attaching to progressively higher levels. Once they reach the surface they can inflate their swim bladders, which occurs within 5 days of fertilization (2–3 days after hatching), at which point they begin food seeking19. Larvae are kept in petri dishes up to day 5, when most have inflated swim bladders. They should then be transferred to a tank, and fed within 48 h. From days 6–15 they can be kept in aerated water with no water exchange (note: reverse-osmosed water must be aerated first), and fed twice daily with a pinch of appropriate food. Overfeeding must be avoided: uneaten food falls to bottom and rots. After 2 weeks the fish can be kept in continuous-flow water systems at a low flow rate so they are not flushed out and fed three times daily with live food. At about 5 weeks dry food is given as well. Once the fish reach 2–3 months they are transferred into larger tanks. Fish grow faster at lower densities. They grow very rapidly in the first 3 months then the growth rate reduces. They reach sexual maturity at about 4 months and will then start breeding. Fish continue to grow until about 18 months.

Initially all gonads develop as ovaries, then in males these begin to develop into testes after 5–7 weeks, maturing by about 3 months. Sexual maturity appears to be related to size, rather than age, when approximately 23 mm long. This typically takes around 75 days but is related to temperature and food availability. The mean life span for zebrafish is 42 months19.

Health and disease

Zebrafish are robust fish but can be affected by a number of diseases that can affect the entire colony, for example velvet disease, fish tuberculosis and nematodes. *Pseudoloma neurophilia* is a common fungal infection in laboratory zebrafish colonies, and the incidence will probably increase as the use of zebrafish in research increases. Appropriate biosecurity measures, husbandry and breeding strategies, and health-screening protocols should be implemented to minimise the effects of these diseases[20].

Rainbow Trout

Rainbow trout are a teleost fish of the salmonid family. They are native to the Pacific drainages of North America, but have been introduced widely around the world for recreation and aquaculture. They are commonly used in ecotoxicology studies. Rainbow trout are very hardy, tolerating a wide range of environments. Some strains are anadromous (live in the ocean returning to spawn in fresh water), whereas others live permanently in freshwater lakes. They grow rapidly, with freshwater strains reaching 4.5 kg and anadromous strains reaching 7.5–10 kg within 3 years6. Rainbow trout live for 5–6 years and can reach 10 kg in weight2.

In the laboratory trout may be held in glass or plastic tanks. Circular tanks help maintain a regular current and uniform distribution of the fish, but square tanks are also acceptable. Tanks are usually 50–60 cm deep. Circulation systems are best. Water can be delivered in circular tanks to the side of the tank so as to create a current, with a drain in the centre of the floor: water then forms a vortex towards the centre that accumulates wastes for easy removal. The drain should be protected by a mesh screen.

Feeding

In the wild, adult trout feed on freshwater shrimp, aquatic and terrestrial insects, molluscs, crustaceans, fish eggs and small fishes. Fry can be weaned onto an artificial diet.

Fry are fed specially prepared starter feeds, starting from when approximately 50% have reached the ‘swim-up’ stage when they begin seeking food. When most fish are actively feeding they should be fed 10% of their weight daily for 2–3 weeks. When the fry are 15–25 mm long feeding is based on published charts, related to temperature and fish size. In the early stages overfeeding must be avoided. As growth continues fish are moved to larger tanks to reduce density6.

Breeding

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Maturation in salmonids is triggered by decreasing photoperiod2, but spawning times vary between strains. Wild rainbow trout usually spawn once yearly, in spring6, but some cultured strains can spawn all year round. Eggs are laid on gravel nests, covered with gravel and left to mature2. Trout rarely spawn naturally outside their native range, and juveniles may need to be obtained from hatcheries6. Photoperiod can be manipulated to produce mature males and females, and embryos produced by artificial fertilization using eggs and milt removed manually from mature fish (males 2–3 years old, females 3–4 years old) under anaesthesia. Hatching depends on temperature, taking approximately 300 degree days2, for example approximately 100 days at 3°C and 21 days at 14.4°C. Trout hatch with a supply of food in the yolk sac, which lasts 2–4 weeks. Once the yolk is absorbed the fish begin actively seeking food. This is known as the swim-up stage.

After hatching, growth is rapid, with males reaching maturity at approximately 2 years and females 3 years.

Health and disease

Rainbow trout may develop a number of infections in the laboratory, including sleeping disease, furunculosis or rainbow trout fry syndrome. The presence of disease may only be identified through an increase in mortality. Fry often carry these diseases subclinically, and outbreaks of disease may not be seen until the animals are stressed by procedures.

Amphibians

The class Amphibia includes a variety of aquatic, semi-terrestrial and terrestrial tetrapods, which have colonized a number of particular habitats successfully. There are about as many amphibian species as mammals[21]. Amphibia are ectothermic, their body temperature being determined by the environment, although they can use behavioural methods and evaporation to control temperature to some extent. This means their metabolic rate falls at low temperatures, allowing them to survive on very little food. There are three orders of amphibian: Apoda, which have no legs and resemble worms or snakes; Urodeles, which includes newts and salamanders, and Anurans, which includes frogs and toads. The Anurans are tail-less apart from larval stages, with well-developed hind limbs adapted for jumping. The head and trunk are fused and the toes are webbed.

Amphibian skin is moist and delicate, and covered in mucus. The skin acts as a barrier to infection and secretes numerous chemicals with protective functions. Their skin secretions are toxic to deter predators, and stressed animals will produce an excess white secretion. This can be irritant to the mucous membranes of the handler, and can also adversely affect the frogs themselves, leading to morbidity. Affected animals should be removed to clean water and the excess secretion removed. Amphibians do not drink, absorbing moisture through their skin, although they also lose water by this route. They cannot concentrate their urine, and are susceptible to dehydration if placed in dry environments.

Amphibians are unusual in that they respire through the skin, as well as through their lungs and mouth. Frogs don't have ribs, so air is pumped into the lungs by the muscles in the mouth. Larval stages respire via gills which degenerate after metamorphosis.

The most common amphibians used in the laboratory are the anurans *Xenopus laevis* (the African clawed frog) and the smaller *Xenopus tropicalis* (the western clawed frog). *Xenopus* are used particularly in studies of developmental biology because they have a large and easily manipulated embryo, with a single pair of adults producing many thousands of offspring with limited genetic variability. *Xenopus* oocytes are used in molecular biology and studies of DNA replication and repair. The first vertebrate to be cloned was an African clawed frog[22], and genetic modification of *Xenopus* embryos is now relatively commonplace. *Xenopus*, like other amphibians, can regenerate lost limbs.

Biology and behaviour

For detailed information on the biology of *Xenopus* species see Tinsley and Kobel[23].

Xenopus originate from sub-Saharan Africa. They have three claws on each hind foot, from which their name is derived: *Xenopus* means ‘strange foot’. They use the claws to tear up large food pieces, then feed by using their hands to push the food into their mouths, where they use a pump mechanism to suck the food in. They are tongueless, toothless and completely aquatic. They have powerful legs and are strong swimmers. They have no ears, but sense movement and vibration via lateral lines running down the length of their body. This skin is smooth, and greenish-grey on their backs with a creamy white underside. Albino varieties also occur. They shed their skin every season, and may eat their own shedded skin.

Wild *Xenopus* are found in swamps and ditches, where the water is cloudy and affords some protection from predators. *X. laevis* occurs in the cooler parts of a wide range of habitats from South Africa to Sudan. A number of subspecies are recognized,

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and most laboratory research has been done on *X. laevis laevis* from South Africa21.

X. laevis are nocturnal and secretive creatures, preferring dark and cloudy water. They communicate by vocalization and maintain dominance hierarchies. Males call to attract females, and females call back in reply. In the laboratory they are often seen hanging seemingly immobile at the water surface with nostrils protruding. They may even call, particularly in the evenings. They are able to adjust their buoyancy so they can hang like this without effort. Captive *X. laevis* can become tame and may take food from a handler's fingers.

X. laevis are robust, and animals can survive and continue to spawn for 15–20 years or more, reaching a length of 12 cm. *X. laevis* are tetraploid, so there are extra copies of many genes that may or may not be functional.

X. tropicalis is found in the tropical forests of West Africa, suggesting they prefer shade and a relatively constant temperature. It is the only species in the *Xenopus* genus to have a diploid genome. Its genome has been sequenced[24], and it is used in studies of genetics. It has a shorter generation time (<5 months), smaller size (4–6 cm body length) and a larger number of eggs per spawn compared with *X. laevis*. See Table 16.3[21,25] for biological data on *X. laevis* and *X. tropicalis*.

Table 16.3 *Xenopus* biological data[21,25].

	X. laevis	*X. tropicalis*
Average size (cm)		
Male	5–10	3.9–4.5
Female	10–15	4.8–5-5
Clutch size	300–1000	1000–3000
Length ofmetamorphosis	2–3 months	1 month
Age tadpoles begin to feed	5–10 days	3 days
Lifespan (years)	15–20	25–30
Temperature range (°C)	18–20	24–26
Feeding frequency (per week)	1–3	2–3
Heart rate/min		
At 2°C	8	No data available
At 25°C	40–60	
Generation time	1–2 years	4–6 months
Chromosome number	18	10

Male *Xenopus* are usually smaller than females, with slimmer bodies and legs. Females are larger, with hip-like bulges above their rear legs. Both males and females have a cloaca, through which the digestive, urinary tract and reproductive systems pass. The cloaca empties through a single opening called the vent. Females have larger ventral flaps, called anal papillae, located immediately above their cloacas. Males have black, spiny nuptial pads on the inner arms and enlarged thumbs to help grip the females during breeding.

Adult *Xenopus* have traditionally been sourced from the wild. As such they are used to natural conditions and transfer to the laboratory may cause significant stress, and they may adapt poorly. In addition they may be carrying parasites or other disease organisms. It is recommended that amphibians for use in the laboratory be purpose-bred.

In the wild, like most amphibians, social behaviour is mainly restricted to the mating season. However, group housing is advisable, as it improves feeding and reduces fear responses. Group feeding promotes feeding, inducing all animals to feed. At very low stocking densities food is frequently not eaten.

Identification

To identify individual animals there are several non-invasive methods. *X. laevis* have unique patterns on their backs, and photographs can be used to differentiate between them. This is more difficult in *X. tropicalis*, for which the markings are less distinct. However, in both species the colour or pattern of the skin, and the pattern of the blood vessels in the toe webs, can be used for identification, either using visual assessment or via electronic scanning devices. Microchip transponders can also be implanted subcutaneously or into the dorsal lymph sac. Ink spots tattooed onto the belly can persist for several years. More invasive methods such as freeze branding or toe clipping are not recommended.

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Husbandry

Laboratory housing for *Xenopus* has to balance the conflict between their natural environment (deep, murky, still water with vegetation or shade to provide cover and protection from predators) and the experimental requirement for easy maintenance and observation, and access to the animals. In addition, there is often no agreed optimum husbandry method. Minimum requirements can be found in reference 26.

The environment in the laboratory should be designed to match their natural habitat as closely as possible. Both species can be kept in similar conditions, with slightly higher temperatures for *X. tropicalis*, but they should not be kept in the same system. Tanks for *Xenopus* should ideally have dark sides, being lit from above, with refuges and some cover. *Xenopus* are found in the wild in deep water, and water that is too shallow may lead to stress. The minimum required depth is between 6 and 12.5 cm depending on the size of the animal[26], but for optimum welfare a depth of 30 or even 50 cm may be preferable[21]. Animals are commonly kept in a water depth of 15 cm with a lid 5 cm above the water surface. *Xenopus* can jump, so tanks should have well-fitting lids, up to 20 cm above water surface to prevent trauma if the animals do jump. Refuges and cover should be provided, using plastic pipes or vegetation. Recommendations for stocking density for *X. laevis* vary from 2 litres per animal to 12 litres per animal, and 0.6–0.8 litres per animal for *X. tropicalis*. Adult animals sourced from the wild may find it difficult to adapt to such intensive systems21.

Xenopus can be kept in static water systems, where water is changed periodically, or recirculating systems, which provide a constant flow of clean water with controlled temperature and composition.

In static systems water should be changed at least weekly. Since regurgitation of food is common if *Xenopus* are disturbed too soon after eating, this should be done at least 3–5 h after feeding. This facilitates removal of food debris. To avoid the risk of regurgitation water can be changed before feeding, which allows feeding in clean water and reduces stress in the post-feeding period, but food debris then remains in the tank for a longer period. In static systems a substrate of gravel is provided to support a culture of bacteria to denitrify the water.

In recirculating systems the water is filtered and aerated, but the flow rate must be low, so as to produce minimal disturbance to the water, as *Xenopus* rarely inhabit flowing water in the wild. Manual removal of debris may still be required.

Changes in temperature need to be gradual to avoid stress, so when changing tanks, frogs must go into clean water of a similar temperature, and handling should be minimal.

Water should be dechlorinated (tap water can be left to stand for 24 h), or treatments added to remove chlorine. In self-contained water treatment systems, salts may be added to maintain conductivity. Water quality, particularly ammonia and the pH level, should be regularly monitored. Ammonia is excreted by amphibians and its concentration will depend on the water handling regime. The level should be kept below 5 mg/l, depending on the pH (potential harm increases as the pH rises)[26].

Frogs are very sensitive to toxic substances and traces of hand lotions or other chemicals on the hands of handlers may kill the animals. Personnel should always wear impervious gloves to handle frogs or change their water. Many factors affect water quality and water-quality parameters should be checked frequently. Table 16.1 lists appropriate water-quality standards for aquatic species.

Xenopus can adapt to a wide range of temperatures, but spawning seems to be optimal at 20°C for *X. laevis* and 24°C for *X. tropicalis*. All aspects of the physiology of *Xenopus* are affected by temperature, for example immunosuppression occurs below 18°C in *X. laevis*[27] and they become less able to withstand stress as temperature falls. At higher temperatures more food is needed as the metabolic rate rises. The optimum is approximately 18–20°C for *X. laevis*, and is higher for *X. tropicalis* (25–30°C)[21,26,28]. Tadpoles prefer slightly warmer temperatures.

For breeding, 'daylight' fluorescent tubes should be used to provide sufficient ultraviolet light to maintain vitamin D levels. A photoperiod of 12 h/12 h or 13 h/11 h light/dark is appropriate26.

Feeding

As juveniles and adults, *Xenopus* are carnivorous scavengers and will eat both dead and live prey and may cannibalise their own tadpoles. They feed under water, detecting prey by vibrations, odour and sight. *Xenopus* are unusual in that they will feed communally on large prey. In nature, group feeding on large items can trigger a 'frenzy', which encourages all individuals to eat. This may not be advantageous in the laboratory, and offering smaller pieces of food may avoid competition and fighting.

Adult *Xenopus* can be maintained on one to three high-protein feeds each week depending on temperature and light level, being given just sufficient food to be consumed within 30–60 min, but so all animals are fed to satiation to avoid competition21. They can be fed pieces of beef heart or fish fillet. Commercial diets are also available. Any remaining food should be removed to prevent

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contamination of water: this is harder with pelleted diets as they disintegrate. Young animals require more frequent feeding: three to four times weekly or even daily.

Tadpoles are filter feeders, eating phytoplankton. They may be fed algae, drops of strained vegetable baby food (green beans or peas), liquified fish food or commercial powdered tadpole food. More food is added only once the previous food has been consumed.

Breeding

Amphibians are unique among vertebrates in that the juvenile and adult forms are different: fish-like tadpoles undergo metamorphosis into the tetrapod juvenile form. Wild *X. laevis* show seasonal cycles in breeding, spawning from early spring to late summer. Females may spawn up to three times in this period.

X. laevis usually takes 1–2 years to reach sexual maturity, but it can take only 6 months post-metamorphosis in the laboratory21. Spawning can be spontaneous but is usually induced in the laboratory by administering one or two injections of human chorionic gonadotropin (HCG), 48 h apart. This allows for year-round breeding. Adults should be in breeding condition (females well-fed and plump, and males with black nuptial pads on the insides of the arms). Male and female can be put together after the second injection into a spawning tank with a perforated plastic base to protect the eggs. The male grips the female during a mating embrace, known as amplexus, then releases sperm over freshly laid eggs. The pair should be left undisturbed in a darkened tank until mating is over. Fertilisation is external. Alternatively, gravid females can be squeezed after injection to extrude mature oocytes. Fertilisation can be done if required by mixing with testicular extract obtained post mortem from hormone-treated males.

Females in good condition can be bred once per month, although an interval of 2–3 months is typical. Females that have not been bred for a period of 4–6 months may produce poor-quality eggs. Males can be bred more frequently.

Eggs hatch after 48 h, then larvae attach to a vertical surface until the yolk sac is absorbed. They are filter feeders and will begin to feed on suspended food particles, at 5–10 days in *X. laevis* and 3–4 days in *X. tropicalis*. They hang, head down, at a 45° angle in the water and use their tails to generate a current in the water to bring their food to them. *Xenopus* tadpoles are quite large compared to other species, and may need to be separated and re-accommodated several times before they metamorphose. Within limits, the speed of metamorphosis is related to the water temperature, but metamorphosis occurs at about 6–8 weeks. Tadpoles must be kept in clean, aerated water free from chemical contaminants, which is changed frequently. Dead or diseased larvae should be removed as soon as possible.

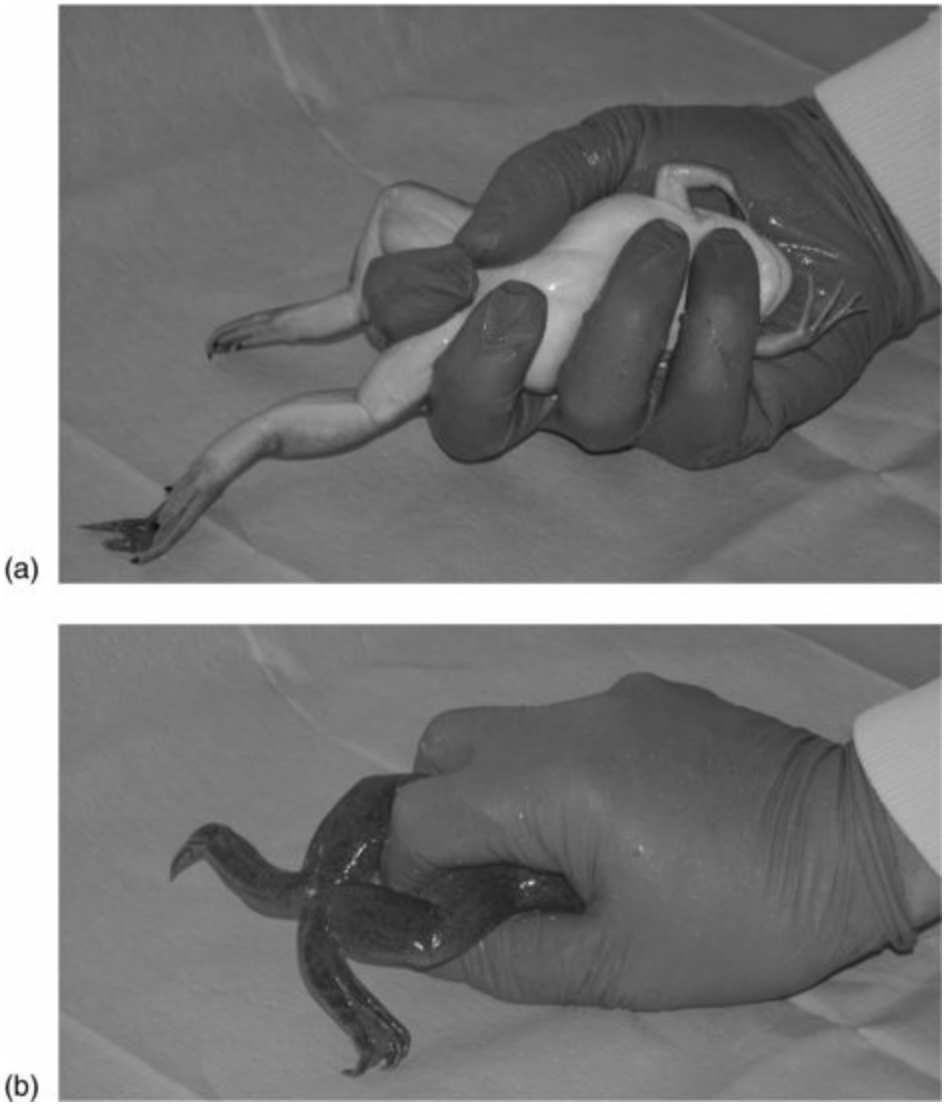
X. tropicalis have a much shorter generation time than *X. laevis*, of only 4 months. Breeding can be induced by hormone injection as for *X. laevis*. Larvae begin feeding from 3 days post-hatching.

Handling

Slippery skin makes handling amphibians difficult. Their powerful hind legs and claws mean they can escape readily and damage the handler. Approach the animal gently. Place one hand over the back of the animal with fingers orientated towards the feet. Place the index finger between the frog's hindlegs, and use the thumb and other fingers to grasp it around the flanks. Grip the animal firmly and remove from water (Figure 16.1). It may be necessary to improve grip in some cases by holding animals with soft moist paper towel or cloth, but the animal must be kept moist at all times.

FIGURE 16.1 Handling frogs.

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A soft net may be the best method to transfer frogs between tanks. Scoop the frog out of the water, keeping a hand over the net to prevent the frog from jumping out.

Anaesthesia

The absorption and excretion of anaesthetic agents is temperature-dependent. Tricaine methane sulphonate (MS222) is frequently used and is usually given by immersion. The animal is placed in a bath of appropriate concentration, and anaesthesia will be induced within 15–30 min. Anaesthesia is judged by loss of righting reflexes and respiratory effort. As the anesthetic level deepens, abdominal respiration is lost, followed by slowing of throat movements, which stop as surgical anaesthesia is reached.

Ketamine (150–250 mg/kg) can be used to provide sedation for minor procedures. See Table 16.4 for information on anaesthetic doses suitable for *Xenopus*.

Table 16.4 Anaesthetic doses for amphibians[29].

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Drug	Dose (mg/l)	Route	Notes
Tricaine methanesulphonate (MS 222)		Immersion	Buffer with $NaHCO_3$ to pH 7–7.5
Adults	500–2000		Induction in 15–30 min; maintain by contact with MS 222 solution; recovery may take 3–6 h; keep animal moist and maintain at 22–26°C
Tadpoles	200–500		
Ketamine	150–250	Intramuscular	Sedation only

Techniques

Administration of substances is often via the dorsal lymph sac. This is a subcutaneous lymph space that connects with the vascular system. Compounds given by this route are rapidly absorbed into the circulation, so this is the usual site for intravenous injection. Insert needle at a shallow angle in the skin of the upper thigh and towards back into the lymph space, or just beneath the skin between the dorsal lateral line stripes.

Intramuscular injections can be given into the thigh muscles. Intraperitoneal injections are given with the frog held on its back, the needle is inserted into the groin area at a shallow angle.

Blood collection in amphibians is often difficult. Small samples can be collected from the toe web. Larger samples may be collected by cardiac puncture post mortem or under terminal anaesthesia.

Surgery

Surgery is often carried out on adult frogs, for example to collect oocytes from gravid females. The skin of frogs contains antimicrobial agents, but it is still necessary to conduct surgery under aseptic conditions, otherwise oocyte quality can be poor[30]. The skin should be prepared prior to surgery by cleansing with dilute povidone iodine solution, which is well tolerated30, the skin covered with a sterile occlusive drape, and surgery carried out using sterile instruments. Skin closure should be carried out using a monofilament suture material. Non-absorbable material may be preferable since soluble sutures often do not last long enough in aquatic environments to support wounds until fully healed. Sutures should be removed as soon as healing has occurred (10–14 days) to prevent reactions to the sutures[31]. Note that reuse of frogs after oocyte collection may be subject to legal constraints.

Health and disease

Pain and stress recognition difficult as animals often appear immobile. Body weight is very variable and depends on hydration status, so body condition may be a better indicator. Prominence of the skeleton and lack of muscles or abdominal content can be indicative of ill health.

The skin should be smooth and slippery with a slight covering of mucus. If it is dry, rough or ulcerated, with an excess mucus covering or shed pieces of skin apparent, there may be ill health.

Numerous diseases can affect *Xenopus*. These are often subclinical and may only become apparent under stress, causing a sudden appearance of signs. *Aeromonas* septicaemia (red-leg) is common. This is normally a commensal organism, but causes problems when animals are stressed. Parasite infections may be found in wild caught animals.

Batrachochytrium dendrobatidis (Bd) is a fungal infection found in laboratory *Xenopus* which does not affect *X. laevis* but affects wild amphibian populations and can cause disease in stressed *X. tropicalis*. Testing for this organism can be done at the European Xenopus Resource Centre (EXRC; see www.port.ac.uk/research/exrc/ for details).

Dehydration is a major cause of morbidity in *Xenopus*, and desiccation must be avoided. The skin is delicate and prone to traumatic injury, which can predispose to infection.

Euthanasia

Schedule 1 permits euthanasia of amphibian by overdose of anaesthetic or concussion. Buffered MS222 can be used at 2–3 g/l.

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Death can take up to 1 h to occur and should be confirmed by pithing. Alternatively an overdose of pentobarbitone can be given into the dorsal lymph sac.

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Glossary

acidosis A reduction in the pH of the blood, commonly due to an increase in blood carbon dioxide level.

adjuvant An added substance which assists or increases the effect of the main ingredient.

alar Related to the wing.

alopecia Hair loss.

anaphylaxis Increased susceptibility to hypersensitivity or infection on second exposure to a substance, characterised by a very rapid reaction.

anorexic Not eating.

anterior An anatomical term meaning towards the front (usually head) end.

apnoea Respiratory arrest.

arrhythmia Irregularity of the heart beat.

ataxia Loss of muscular coordination.

atopy A form of hypersensitivity, where there is a tendency to develop conditions such as hay fever, asthma and eczema.

axenic Animals that are free from detectable microorganisms.

barbering The chewing and nibbling of whiskers and fur by one rodent to exert dominance over another.

calculi Stones formed from mineral deposits, usually in the kidneys or bladder.

candling A technique involving shining a bright light through an (avian) egg shell to evaluate the embryo and blood vessels.

cardiac tamponade Effusion of blood or fluid into the pericardium which prevents the heart from being able to fill effectively, thereby causing circulatory failure.

caudal An anatomical term meaning towards the tail.

chromodacryorrhoea A non-specific indicator of stress in rats characterised by red staining on the face and nose caused by excess tear production overflowing onto the face.

cloned animal An animal produced by inserting nuclear material from one cell into another by nucleus transfer. The offspring are genetically identical to the parent.

conspecific Another animal of the same species.

coprophagy The re-ingestion of faeces carried out by rodents and lagomorphs to allow nutrients released during microbial digestion in the hindgut to be absorbed.

cranial An anatomical term meaning towards the head.

crepuscular Active at dawn and dusk.

cyanosis Bluish coloration of the skin and mucous membranes due to poor blood oxygenation.

cystocentesis Sampling urine by passing a needle through the abdominal wall into the lumen of the bladder (under aseptic conditions).

decerebration Removal or separation of the cerebral cortex, leaving the brain stem intact. Such animals are considered to be insentient but are still alive.

diuretic Promoting the excretion of urine.

dorsal An anatomical term meaning towards the back.

dystocia Difficulty in giving birth.

dysuria Difficulty or pain on urination.

effusion Escape of fluid from blood vessels or lymphatics into a body cavity.

embolism The passage of a blood clot or other particulate matter in the bloodstream to a site distant from the site of origin, causing blockage of a vessel.

emesis Vomiting.

endotoxaemia The presence of endotoxin (bacterial products) in the blood, which frequently results in shock, due to vasodilation.

epidemic A disease affecting a large proportion of individuals in a colony simultaneously.

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epistaxis Bleeding from the nose.

exudate The extravasation of fluid and/or cells from the blood into the tissues, any body cavity or the surface, usually due to inflammation.

genetically altered animal An animal in which the heritable DNA has been manipulated intentionally. This includes genetically modified animals and harmful mutants.

genetically modified animal An animal in which there has been insertion or deletion of DNA in a way that does not occur naturally.

genotype The genetic makeup of an animal.

gnotobiotic Animals that harbour known microorganisms.

haemolysis The splitting or disintegration of red blood cells resulting in the release of haemoglobin.

harmful mutant An animal in which the genome has been altered by mutation or chromosomal changes, without insertion or deletion of DNA, with resulting potential for harm.

hepatotoxin A compound that is toxic to the liver.

hypercapnia A high level of carbon dioxide in the blood.

hypertonic One of two solutions, which has the higher osmotic pressure.

hypnotic As narcotic. A drug that induces sleep.

hypoglycaemia A low blood glucose level.

hypotonic One of two solutions, which has the lower osmotic pressure.

hypoxia A low blood oxygen level.

iatrogenic Caused through medical intervention.

infarction Necrosis of part of an organ or tissue due to blockage of its arterial supply, by a thrombus or embolus in the end arteriole.

isotonic Two solutions having equal osmotic pressure.

laparoscopy Examination of the abdominal cavity usually via the passage of an endoscope through a keyhole incision, for diagnosis or to perform surgical procedures.

laparotomy A surgical procedure involving incision into the abdominal cavity.

lordosis A position adopted by female animals in oestrus to facilitate copulation, with the back arched and the tail held to one side.

meconium The first intestinal discharge from a neonate.

morbidity The proportion of a colony infected by a disease.

mortality The proportion of individuals affected by a disease which die from that disease.

mutagenesis Exposing animals to chemicals, viruses or radiation to increase the rate of gene mutation.

narcotic A substance that induces sleep.

nephrotoxin A substance that is toxic to the kidney.

nystagmus A repetitive movement of the eyeballs, consisting of a slow movement in one direction followed by a rapid return.

phenotype The physical characteristics of an animal.

pheromone Hormone-like substance secreted by one animal that causes behavi- oural, physiological or endocrine changes in another.

phlebitis Inflammation of a vein.

posterior An anatomical term meaning towards the rear.

ptyalism Excess saliva production and dribbling

pyrexia Fever.

rostral An anatomical term meaning towards the nose or beak, used for describing structures on the head.

ruminal tympani Distension of the forestomach (rumen) with gas.

sedative A drug that produces a calming effect and drowsiness.

sentient Capable of feeling, perceiving sensations.

skin tenting A phenomenon, usually caused by dehydration, in which a fold of skin remains raised for several seconds after being pinched.

specific-pathogen-free Animals free from particular (named) microorganisms.

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stereotypy The performance of unusual motor acts, repeatedly and often invariably, which serve no apparent purpose. Often indicative of an inadequate environment.

subclinical A disease causing no clinical signs. Often occurs prior to the appearance of manifest symptoms.

syncope A sudden fall in blood pressure or heart failure leading to cerebral anaemia and loss of consciousness.

tachycardia Rapid heart beat.

teratogenesis The production of abnormalities in a fetus due to exposure of the dam to physical or chemical insult.

therapeutic index The ratio between the lethal dose of a drug and its effective clinical dose.

thigmotaxis Wall-hugging behaviour.

thrombosis The inappropriate formation of a blood clot.

tranquillizer A drug that produces a calming effect without drowsiness

transgenic An animal into which additional genes have been inserted.

transudate The passage of fluid into the tissues or a body cavity, usually passively without inflammation.

urticaria Nettle rash.

ventilation A term used to describe forced breathing, by the application of positive pressure to the airways (intermittent positive pressure ventilation).

ventral An anatomical term meaning towards the belly.

xiphisternum Cartilaginous process on the caudal end of the sternum.

zoonosis A disease communicable from animals to humans.

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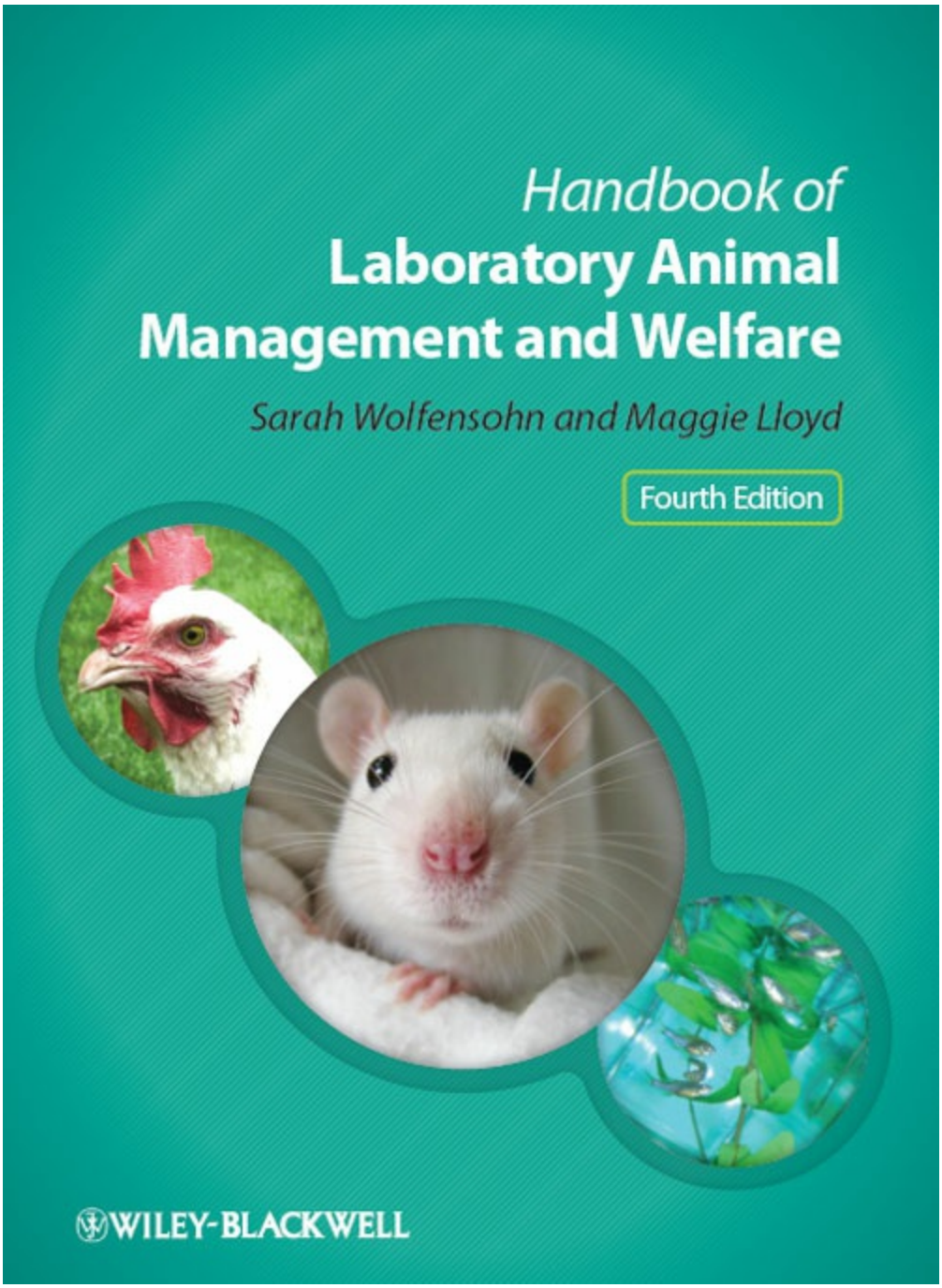
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