European Commission

Joint Research Centre

Laboratory animal science and technology

Entry level

Stephen Barnett, Ken Applebee and David Anderson

2026

This document is a publication by the Joint Research Centre (JRC), the European Commission's science and knowledge service. It aims to provide evidence-based scientific support to the European policymaking process. The contents of this publication do not necessarily reflect the position or opinion of the European Commission. Neither the European Commission nor any person acting on behalf of the Commission is responsible for the use that might be made of this publication. For information on the methodology and quality underlying the data used in this publication for which the source is neither Eurostat nor other Commission services, users should contact the referenced source. The designations employed and the presentation of material on the maps do not imply the expression of any opinion whatsoever on the part of the European Union concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

Joint Research Centre
https://joint-research-centre.ec.europa.eu

JRC132507

Print	ISBN 978-92-76-99198-4	doi:10.2760/205643	KJ-NA-31-396-EN-C
PDF	ISBN 978-92-76-61970-3	doi:10.2760/674109	KJ-NA-31-396-EN-N

Luxembourg: Publications Office of the European Union, 2026

© European Union, 2026

The reuse policy of the European Commission documents is implemented by the Commission Decision 2011/833/EU of 12 December 2011 on the reuse of Commission documents (OJ L 330, 14.12.2011, p. 39). Unless otherwise noted, the reuse of this document is authorised under the Creative Commons Attribution 4.0 International (CC BY 4.0) licence (https://creativecommons.org/licenses/by/4.0/). This means that reuse is allowed provided appropriate credit is given and any changes are indicated.

For any use or reproduction of photos or other material that is not owned by the European Union permission must be sought directly from the copyright holders (see acknowledgements section).

How to cite this report: Barnett, S., Applebee, K. and Anderson, D., *Laboratory animal science and technology*, Louhimies, S., Deceuninck, P., eds., Publications Office of the European Union, Luxembourg, 2026, https://data.europa.eu/doi/10.2760/674109, JRC132507

Contents

Foreword 10

Introduction 11

Acknowledgements 12

1. EU legislation on the protection of animals used for scientific purposes 13

1.1. General introduction 13

1.2. Animal use in the EU 14

1.3. Scope of the Directive 15

1.3.1. Establishment 15

1.3.2. Project 16

1.3.3. Procedure 17

1.3.4. 'Thresholds' 18

1.3.5. Severity classification 18

1.3.6. Genetically altered animals 19

1.3.7. Support personnel within an establishment 19

1.4. Animal welfare bodies 20

1.5. Animal care responsibilities 21

1.6. Fate of animals 22

1.7. Other related legislation 22

1.7.1. Transport 23

1.7.2. Animal health 23

1.7.3. Veterinary legislation 23

1.7.4. Medicines legislation 23

1.7.5. Genetic modification legislation 23

1.7.6. Convention on International Trade in Endangered Species of Wild Fauna and Flora 24

1.7.7. Wildlife legislation 24

1.7.8. Human safety and health 24

2. Application of the severity assessment framework 28

2.1. General introduction 28

2.2. Project and procedures 29

2.2.1. Project 29

2.2.2. Procedure 29
2.3. Thresholds of severity 29
2.3.1. Lower threshold 29
2.3.2. Upper limit 30
2.4. Assignment of severities 30
2.4.1. Prospective severity classification 30
2.4.2. Assessment of actual severity 32
2.4.3. Consistency in the assessment of severity 33
2.5. Summary 34
3. Ethics, the Three Rs and the culture of care 36
3.1. Introduction 36
3.2. Ethics 36
3.3. Public attitude to the use of animals in science 36
3.4. Humane experimental technique: The Three Rs 37
3.4.1. Examples of replacements 39
3.4.2. Examples of reduction 39
3.4.3. Examples of refinement 40
3.4.4. Environmental enrichment 42
3.5. Culture of care 46
3.6. Sources of information 47
4. Maintaining health status 50
4.1. Introduction 50
4.2. Causes of disease 50
4.2.1. Infectious disease 51
4.2.2. Non-infectious disease 52
4.3. Controlling the passage of infectious agents into and out of animal facilities 53
4.3.1. Bio-exclusion barriers 54
4.3.2. Embryo transfer rederivation 54
4.3.3. Isolators 54
4.3.4. The specified pathogen-free facility 55
4.3.5. Entering the facility 57

4.3.6. Bio-containment 61
4.4. Animal health screening programmes 62
4.4.1. Collecting samples for health screening 62
4.5. Inspecting individual animals for their health and welfare status 62
4.5.1. Detailed health inspection 64
4.5.2. Example of a non-mammalian species: zebrafish 68
4.5.3. Stress 70
4.5.4. Pain 70
4.6. Action to be taken after completing the welfare inspection 71
5. Housing and environment 74
5.1. Introduction 74
5.2. Animal enclosures and housing 74
5.2.1. Enclosure design space and stocking density 74
5.3. Enclosure material 77
5.4. Development of enclosure design 80
5.4.1. Arrangement of cages 81
5.4.2. Pens 81
5.5. Example of a non-mammalian species Zebrafish 83
5.6. Environment 84
5.6.1. Other animals 85
5.6.2. Heating, ventilation and air conditioning system (HVAC) 86
5.6.3. Temperature 88
5.6.4. Humidity 90
5.6.5. Light 91
5.6.6. Noise 94
5.6.7. Vibration 96
5.7. Example of a non-mammalian species Zebrafish 96
5.8. Monitoring the environment 97
5.8.1. Measuring temperature and humidity 97
6. Routines, husbandry and hygiene 101
6.1. Introduction 101

6.1.1. Routines ... 101
6.1.2. Standard operating procedures ... 102
6.2. Hygiene ... 107
6.2.1. Cleaning cages and pens ... 108
6.2.2. Cage wash area ... 111
6.3. Bedding and nesting ... 114
6.3.1. Characteristics of an ideal bedding material ... 114
6.3.2. Examples of bedding and nesting materials ... 115
6.4. Sterilisation and disinfection ... 121
6.4.1. Sterilisation and disinfection techniques ... 121
6.4.2. Definitions used in connection with sterilisation and disinfection ... 121
6.4.3. Sterilisation ... 123
6.4.4. Disinfection ... 128
6.4.5. Heat ... 129
6.4.6. Chemical disinfection ... 129
6.4.7. Properties and uses of disinfectant groups ... 130
7. Nutrition ... 134
7.1. Introduction ... 134
7.2. Nutrients ... 134
7.2.1. Proteins ... 134
7.2.2. Carbohydrates ... 135
7.2.3. Lipids/fats ... 136
7.2.4. Vitamins ... 137
7.2.5. Minerals ... 138
7.3. A balanced diet ... 138
7.3.1. Formulation of diets ... 138
7.3.2. Contribution of the dietary ingredients to the nutritional value ... 139
7.3.3. Diets ... 139
7.4. Forms of diet ... 140
7.4.1. Pelleted diet ... 140
7.4.2. Powdered diet ... 141

7.4.3. Mash ... 141
7.4.4. Additional processed diets ... 142
7.4.5. Fresh food ... 142
7.4.6. Special nutritional requirements ... 143
7.5. Presentation of diet ... 143
7.6. Diet quality ... 145
7.6.1. Chemical deterioration ... 145
7.6.2. Contamination by organisms ... 146
7.6.3. Storage of diet ... 146
7.7. Food pests ... 147
7.7.1. Moulds ... 147
7.7.2. Flour mite ... 148
7.7.3. Flour moth ... 148
7.7.4. Grain weevil ... 149
7.7.5. Sawtoothed grain beetle ... 149
7.8. Water ... 150
7.8.1. Methods of presenting drinking water ... 150
8. Breeding common laboratory animals ... 155
8.1. Introduction ... 155
8.1.1. Stocking densities ... 155
8.2. Glossary of breeding terms ... 155
8.3. Breeding systems ... 158
8.3.1. Criteria for the choice of system ... 158
8.4. Breeding systems for selected species ... 159
8.4.1. Introduction ... 159
8.4.2. The mouse ... 160
8.4.3. The rat ... 162
8.4.4. The Syrian hamster ... 162
8.4.5. The guinea pig ... 163
8.4.6. The rabbit ... 164
8.4.7. The ferret ... 165

8.4.8. The cat ... 166
8.4.9. The dog ... 166
8.5. Example of a non-mammalian species ... 168
8.5.1. Notes on zebrafish health, by Nicola Goodwin ... 168
8.6. Genetically defined laboratory animals ... 169
8.6.1. Inbreeding ... 169
8.6.2. Non-inbreeding techniques ... 171
8.7. Specific genetic models ... 172
8.7.1. Co-isogenic strains ... 172
8.7.2. Congenic strains ... 172
8.7.3. Recombinant inbred strains ... 172
8.7.4. Genetic mutants ... 173
8.8. Breeding records and productivity measurements ... 173
8.9. Selection of breeding stock ... 174
8.10. Weaning ... 175
8.11. Pre-weaning mortality ... 175
8.12. Economic breeding life ... 175
8.13. Productivity ... 175
8.14. Physical development of young animals ... 177
8.14.1. Mice and rats ... 177
8.14.2. Syrian hamsters ... 179
8.14.3. Guinea pigs ... 179
8.14.4. Rabbits ... 179
8.14.5. Carnivores (cats, dogs and ferrets) ... 180
9. Genetically altered animals ... 184
9.1. Introduction ... 184
9.2. Uses for genetically altered animals ... 184
9.3. Genome editing in the mouse: an introductory overview ... 184
9.4. Monitoring ... 189
10. Marking and identification of animals ... 191
10.1. Introduction ... 191

10.2. Legal considerations ... 191
10.3. Selecting the most suitable method of identification ... 191
10.4. Commonly used methods of identifying laboratory animals ... 192
10.4.1. Non-invasive methods ... 192
10.4.2. Invasive methods ... 195
11. Minimally invasive procedures without anaesthesia and the welfare concerns associated with them ... 202
11.1. Introduction ... 202
11.2. Preparatory refinement ... 202
11.2.1. Transport ... 202
11.2.2. On arrival ... 203
11.3. Minimally invasive procedures: techniques ... 204
11.3.1. Administration of experimental substances ... 204
11.3.2. Routes of administration ... 205
11.4. Collection of samples ... 209
11.4.1. Tissue biopsy ... 209
11.4.2. Blood ... 209
11.4.3. Collecting samples of faeces and urine ... 211
11.4.4. Measurement of food and water intake ... 211
11.4.5. Metabolism cages ... 211
11.5. Post-procedural care ... 213
12. Euthanasia ... 216
12.1. Introduction ... 216
12.2. Factors that affect the choice of method of euthanasia ... 216
12.3. Legal considerations ... 217
12.4. Methods of euthanasia permitted by the Directive ... 219
12.4.1. Physical methods ... 219
12.4.2. Chemical methods ... 221
12.5. Recognition of signs of death ... 223
13. Handling and sexing ... 226
13.1. Introduction ... 226
13.2. General principles of animal handling and sexing ... 226

13.3. Techniques for individual species 227
13.3.1. The mouse 227
13.3.2. The rat 231
13.3.3. The Syrian hamster 235
13.3.4. The guinea pig 236
13.3.5. The rabbit 237
13.3.6. The ferret 241
13.3.7. The cat 243
13.3.8. The dog 244
13.4. Example of a non-mammalian species: zebrafish by Nicola Goodwin 245
13.4.1. Handling 245
13.4.2. Sexing 246
14. Safety and health in the laboratory animal facility 248
14.1. Introduction 248
14.2. Legal considerations 248
14.3. Safety and health information 249
14.4. General safety in a laboratory animal facility 249
14.4.1. Safe work practice 249
14.4.2. Safety directly associated with animals 250
14.5. Biological hazards 251
14.5.1. Zoonoses 251
14.5.2. Disposal of biological waste 252
14.6. Manual handling 253
14.6.1. Repetitive strain injuries 253
14.7. Hazard warning signs 254
14.8. Chemical hazards 255
14.8.1. Safe use of chemicals 255
14.8.2. Spillage of chemicals 257
14.8.3. Transporting chemicals 257
14.9. Fire 258
14.10. Electrical hazards 259

14.11. Accident procedures 261

15. Correlation of the Manual with EU Education and Training Framework Modules and Learning Outcomes 264

References and further reading 266

List of abbreviations 272

List of figures 274

List of tables 281

Foreword

Welcome to the manual Laboratory Animal Science and Technology, an essential resource designed to help you develop the expertise, commitment and ethical grounding necessary to excel in your new role. As you begin this journey, you are a key player at the forefront of animal use in research. Your dedication not only positively impacts the lives of animals under your care, but also enhances the integrity and reliability of scientific research, benefiting humans, animals and the environment.

Recognised as sentient beings, animals entrusted to our care must be treated with respect, safeguarding their dignity and welfare within the context of scientific use. The principles of the Three Rs – Replace, Reduce, and Refine the use of animals in science form the foundation of this manual. These principles are not merely a legal obligation in the EU; they also reflect an ethical commitment to continually improve the conditions and experiences of animals for as long as their use continues, while working towards the Union's goal of full replacement.

This manual will serve as your comprehensive guide and trusty companion. It is a common introductory reference for laboratories across the EU, providing the tools to develop high standards of competency, compassion and care. Each chapter guides you through essential topics, using commonly agreed learning outcomes that build your knowledge and skills progressively. At the end of each chapter, you'll find summaries of key elements aligned with the Three Rs, along with questions to reinforce and test your understanding.

I hope this manual acts as both an educational guide and a step towards furthering a culture of care in laboratory animal welfare. Your role as a caretaker or technician is vital in achieving this goal. 'Caring for animals, aiming for better science' is more than a slogan; it's our shared mission. With knowledge, experience and skills, you will gain the confidence to question current practices, thereby fostering a positive institutional culture of challenge that strives for new opportunities to implement the Three Rs, and higher standards of animal welfare and scientific quality.

Susanna Louhimies

Policy Coordinator

European Commission

Introduction

This book has been written to support employees starting careers as animal carers in laboratory animal establishments. Animal carers (also known as animal caretakers or technicians) play an essential role in ensuring the efficient operation of a laboratory animal establishment. It is important, therefore, that an effective training programme exists to enable animal carers to fulfil their role. The EU has developed a syllabus entitled the EU Educational and Training document which lists the Learning Outcomes that individuals must achieve to become qualified carers. This book covers many of those Learning Outcomes and it is hoped that it will provide a good understanding of how laboratory animal establishments function.

In addition to this book, further education and training will be gained from other sources, for instance, tutors and experienced colleagues in the workplace. It is not possible to become a skilled animal carer by just reading alone, developing practical skills is of at least equal importance and those can only be gained by working alongside experienced workers.

Laboratory animal establishments must provide a stable environment that satisfies the animals behavioural, physiological and welfare needs in a way that enables scientists to carry out their research work and produce reliable experimental results. The day-to-day care of the animals in establishments is the responsibility of animal care staff and this book explains the work to new employees.

In the EU, scientific procedures involving animals are subject to legislation (Directive 2010/63/EU). As all employees working with laboratory animals must be familiar with the law so **Chapter 1** outlines the key provision of the Directive. Employees should also be familiar with the implementation of the Directive operating in the Member State they are working in.

Throughout the book the authors emphasise the importance of animal welfare, the culture of care and the relevance of applying Replacement, Reduction and Refinement (the Three Rs) to all aspects of laboratory animal work. These topics are covered in **Chapter 3**.

The remainder of the book covers the other main aspects of the work of laboratory animal carers, e.g. housing and environment, hygiene, feeding, breeding and handling and sexing. At the end of each chapter are suggestions for further reading and a few self-assessment questions.

The internet is a major source of information. However, because anybody can upload data onto the web, care must be taken to critically evaluate articles before accepting what it says. A good practice is to look at where the author(s) come from. If it is from a respected governmental or national organisation, University, research institute or similar, the information can generally be relied on. If the author has no affiliation and no references supporting the article further evidence should be sought before relying on what the article says.

Guidance can always be sought from experience colleagues in the workplace, if in doubt ask, it is a great way to learn!

Acknowledgements

We have consulted a number of people in the production of this book. They include: Tina O'Mahony, Nicky Gent, Gary Childs, Sue Applebee, Sara Wells, Alison Hopkins and Steven Cubitt. Graham Cocks was responsible for much of the GAA chapter and Nichola Goodwin was responsible for all the material on zebrafish.

Several people have granted us access to their departments to take photographs and other companies or individuals have allowed to use their own photographs.

We are grateful to Wendy Jarrett, Chief Executive of Understanding Animal Research for permission to use the cover image.

Others who should be mentioned are: Jasmine Barley, Roger Francis, Brian Lowe, Allan Thornhill, Wendy Steel, Sarah Lane, Gemma Marshall, James Wilson, James Bussell, Jim Scott, John Waters, Mark Gardner, Sandra Batty, Nicky Windows, Greg Spencer, Roy Sutcliff. All those mentioned above have been a great help in completing this book and we are grateful for their help.

We are grateful to the individuals and organisations who have allowed us to reproduce their images and tables in this book. Their names are indicated under their contributions.

1. EU legislation on the protection of animals used for scientific purposes

This chapter provides you with an introduction to the EU laws that govern the protection of animals used for scientific purposes. It provides information on the regulatory framework within which projects involving animals are authorised and the legal responsibilities of the people involved, that is, those carrying out procedures on animals, those designing procedures and projects, those taking care of animals and those killing animals. Although national legislation may differ in how the European law has been implemented in each EU Member State, the general requirements and objectives will have been applied (module 1).

1.1. General introduction

Legislation in the EU is embodied in regulations and directives.

Regulations set specific obligations that the Member States of the EU are required to follow exactly. One example of a regulation is the legislation on the transport of animals, which sets out specific requirements for transport that must be followed, for example maximum journey times and specified rest periods.

With regard to the legislation on the protection of animals for scientific procedures, this is contained in Directive 2010/63/EU ('the Directive' from hereon) which includes the requirements for the accommodation, care and use of animals bred, supplied for and used in scientific procedures.

Directives differ from regulations in that, although the obligations set out in a directive need to be delivered, flexibility is allowed in how these are achieved. Each Member State incorporates the Directive into national legislation; as a consequence, different structures may emerge. For example, although all persons have to be trained and competent to carry out scientific procedures on animals, how this is achieved among Member States differs. In some countries, individuals require a personal authorisation, but in others the establishment is responsible for ensuring the appropriate training and competence of individuals.

Under the EU legislation, the Directive 2010/63/EU, all establishments where animals are bred or supplied for scientific use, and all establishments where animals are used in scientific procedures, must be authorised by and registered with the relevant authority within the Member State.

Each establishment needs to meet the required standards of accommodation and care, have an Animal Welfare Body (AWB) and have a designated veterinarian (DV) to advise on animal welfare, care and use.

All vertebrate animals, including certain immature forms, and a group of invertebrates (cephalopods, which includes squid and octopus) are included in the scope of the Directive, as is the creation of genetically altered animals (GAAs).

The use of non-human primates is permitted, subject to additional control measures.

The details of all scientific procedures using animals (experiments) must be specified in the authorised project. The authorisation includes animal numbers, the species to be used and the procedures to be applied, including measures to minimise suffering.

All personnel caring for animals, designing procedures and projects, performing procedures on animals and killing animals need to be trained and competent.

Establishments must be inspected. The frequency of inspection is risk based, but a minimum of one third of all user establishments must be inspected each year, and all those using, breeding or supplying non-human primates are to be inspected annually.

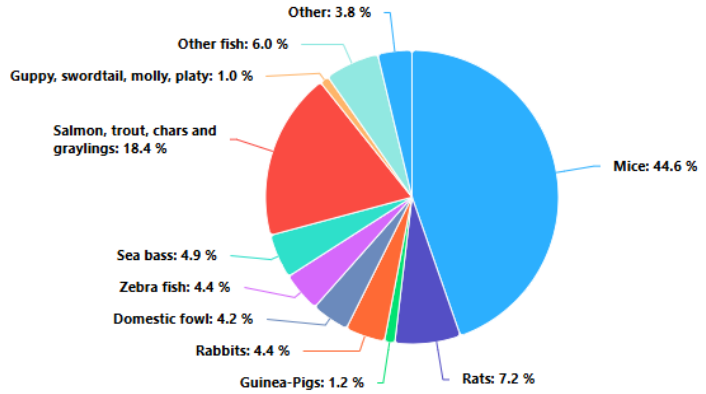
Failure to comply with the Directive can result in administrative (e.g. revocation of project authorisation) or legal action.

1.2. Animal use in the EU

The number of animals used in the EU is reported annually: in 2023, just under 8 million animals were used for the first time in the EU and Norway, with mice (44.6%) and fish (34.7%) the most commonly used animals. A total of 4,392 non-human primates (0.06%) were used for the first time (**Figure 1.1.** Percentages of animals used in the EU and Norway for the first time in 2023).

A further 850 000 animals were used in the creation and maintenance of GAA lines, almost all of which were mice or zebrafish.

Figure 1.1. Percentages of animals used in the EU and Norway for the first time in 2023

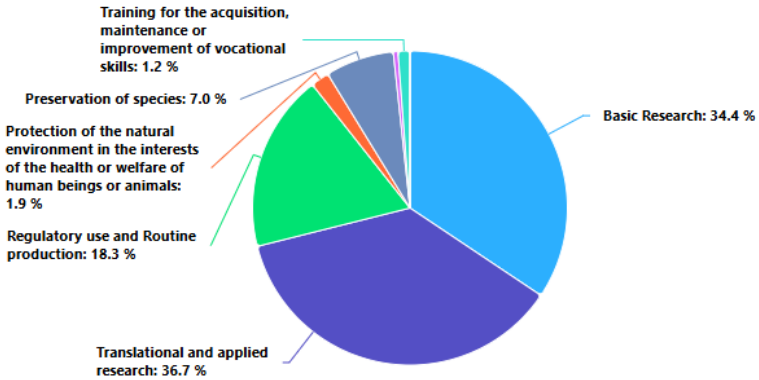


Source: Animal Use Reporting – EU System (ALURES) EU statistical database, 2023.

The purposes for which animals are used are also available from the published reports (**Figure 1.2.** Purposes of animal use in the EU and Norway in 2023).

Most animals are used for basic or translational and applied research. Just under 20% are reported as being used for regulatory use and routine production.

Figure 1.2. Purposes of animal use in the EU and Norway in 2023



Source: ALURES EU statistical database 2023

NB: Further information may be found at https://environment.ec.europa.eu/topics/chemicals/animals-science/statistics-and-non-technical-project-summaries_en

1.3. Scope of the Directive

The Directive applies to all live non-human vertebrates, including independently feeding larval forms (e.g., with fish, the time at which they fall within scope differs between species and varies with the temperature at which they are kept; as an example, the commonly used zebrafish is considered in the scope of the Directive from five days post fertilisation when kept at 28°C) and fetal forms of mammals from the last third of their normal development (e.g. mouse fetus from day 14 of pregnancy, assuming a 21-day gestation).

Project authorisation is also required for the creation of a new genetically altered lines of animals, and to breed and maintain lines that may express a harmful phenotype, for example lines that have impaired immune systems, rendering the animals at risk of infection.

Moreover, the Directive mandates that the commonly used laboratory species are purpose-bred for use in scientific procedures, although exemptions are permissible provided there is scientific justification, for example studies of mice in the wild.

The Directive does not apply to the following:

1. non-experimental agricultural practices (e.g. disbudding calves);
2. non-experimental clinical veterinary practices (e.g. clinical treatment of pets, blood sampling for disease diagnosis);
3. veterinary clinical trials required for the marketing authorisation of veterinary medicines (this is the very last stage of testing new drugs before they are licensed, by which time there will already be test data on safety and efficacy gathered under a project authorisation);
4. practices undertaken for the purposes of recognised animal husbandry (e.g. vaginal swabbing (mice/dogs) or blood sampling (dogs) to determine stage of oestrus and optimum time for mating);
5. practices undertaken for the primary purpose of identification (e.g. ear notching in mice);
6. practices not likely to be above the lower threshold (i.e. not likely to cause 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), for example observational behavioural studies or assessing body composition by non-invasive measures with minimal restraint.

1.3.1. Establishment

Each establishment where animals are housed for scientific purposes is authorised by and registered with the **competent authority**. An establishment can be authorised as a **breeder** (breeds animals with a view to their use in science, either in projects/procedures or for the use of their tissues/organs), a **supplier** (supplies animals with a view to their use in science, either in procedures or for use of their tissues/organs) or a **user** (uses animals in procedures), or an establishment can be authorised for any combination of the three.

Within each establishment, a specified person has overall responsibility for ensuring compliance with the Directive.

Each establishment shall meet the standards of accommodation and care set out in an Annex to the Directive which gives species-specific requirements for animal care including the environment and

minimum enclosure dimensions. The establishment must provide and maintain facilities and equipment suited to the species to be housed and required for the proposed scientific work.

The person responsible for compliance is responsible for ensuring that procedures are carried out as effectively as possible, and that the **Three Rs** (Replacement, Reduction and Refinement) are promoted and implemented.

There are also obligations to ensure that there is a sufficient number of staff on-site to provide appropriate monitoring and care, and that such staff are adequately educated, trained and competent before carrying out procedures, designing procedures and projects, taking care of animals and killing animals.

After individuals have completed suitable education and training, there is an obligation to supervise them as appropriate, until they are assessed as competent to work unsupervised. There are also ongoing training obligations to ensure that those with animal care responsibilities remain up to date.

1.3.2. Project

All scientific procedures on animals must be authorised within a project.

According to the Directive, a project means a programme of work having a defined scientific objective and involving one or more procedures.

Projects are carried out within an establishment, although exemptions can be given when there is a specific need, for example, working with wildlife or using a commercial farm.

Projects can vary in size and complexity, from the work of a single scientist conducting a single blood-harvest procedure in a single species to an entire department's drug discovery programme, which involves many scientists, multiple complex procedures and a wide range of species.

Projects can be authorised for the following purposes only (Article 5):

a) basic research;

b) translational or applied research for the following purposes only:

c) the avoidance, prevention, diagnosis or treatment of disease, ill health or other abnormality or their effects in human beings, animals or plants;

d) the assessment, detection, regulation or modification of physiological conditions in human beings, animals or plants;

e) the welfare of animals and the improvement of the production conditions for animals reared for agricultural purposes;

f) for any of the aims in point (b) in the development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs and other substances or products;

g) protection of the natural environment in the interests of the health or welfare of human beings or animals;

h) research aimed at preservation of the species;

i) higher education, or training for the acquisition, maintenance or improvement of vocational skills;

j) forensic inquiries.

For permission to conduct a project, the applicant applies to the competent authority/ies responsible for project evaluation and authorisation. This application includes information on the scientific objectives and on all animal use. All procedures have a severity assigned, which reflects the most severe potential consequences, adverse effects, that an individual animal could experience. The application also includes information on actions to be taken to minimise these and identifies humane endpoints. The application indicates how the Three Rs will be addressed and includes a non-technical project summary providing information in lay (non-scientific) language on the benefits and harms of the project and the application of the Three Rs. Non-technical project summaries are published on the EU website.

The application is reviewed through a project evaluation process. This evaluates the objectives and predicted benefits of the scientific programme, compliance with the Three Rs and the appropriateness of the severity classification. A harm-benefit analysis is made to assess whether the harm to the animals is justified by the expected outcomes.

Projects are authorised for a maximum of five years.

The project holder is responsible for compliance with the authorised project, and is responsible for providing statistical information annually on animal use, including the actual severity experienced by each animal.

1.3.3. Procedure

Within a project, animals are used in procedures.

The Directive defines a procedure as any **use** of an animal:

- that is **invasive** (e.g. an injection of a test substance) or **non-invasive** (e.g. addition of a test substance in the normal diet);
- **for experimental or other scientific purposes** (the specific purposes are given above);
- **with known** (e.g. blood harvest for *in vitro* use) **or unknown outcome** (e.g. injection of a test substance);
- **that may cause the animal a level of 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**;
- including **any course of action intended, or liable, to result in the birth or hatching of an animal or the creation and maintenance of a genetically modified animal line in any such condition**, but excludes the killing of animals solely for the use of their organs or tissues.

Procedures may be simple or complex, depending on the purpose.

The purpose may be achieved by using a simple single-step procedure (e.g. withdrawal of blood to be used in *in vitro* studies), but much more commonly requires a number of steps (multi-step procedure) used in combination to achieve a single outcome, and often requires the use of the same animal (e.g. antibody production would generally require a number of injections to stimulate antibody production, followed by a number of blood samples to monitor and harvest the antibodies).

1.3.4. 'Thresholds'

The Directive gives a legal definition of the **threshold** above which a project authorisation is required to permit the scientific investigation to continue. This is set as a level of 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.

When performed competently, penetration of the skin by a needle is likely to cause transient pain of low intensity. (This interpretation does not include consideration of the type of substance injected or the potential adverse effects of the substance following injection.)

As the 'threshold' in the Directive has only this one example, there is often much discussion over other 'thresholds' for suffering, distress and lasting harm resulting from non-invasive techniques, for example single housing of social species, restriction of food and water, feeding of modified diets and behavioural testing, and when these can be considered equivalent to the introduction of a needle.

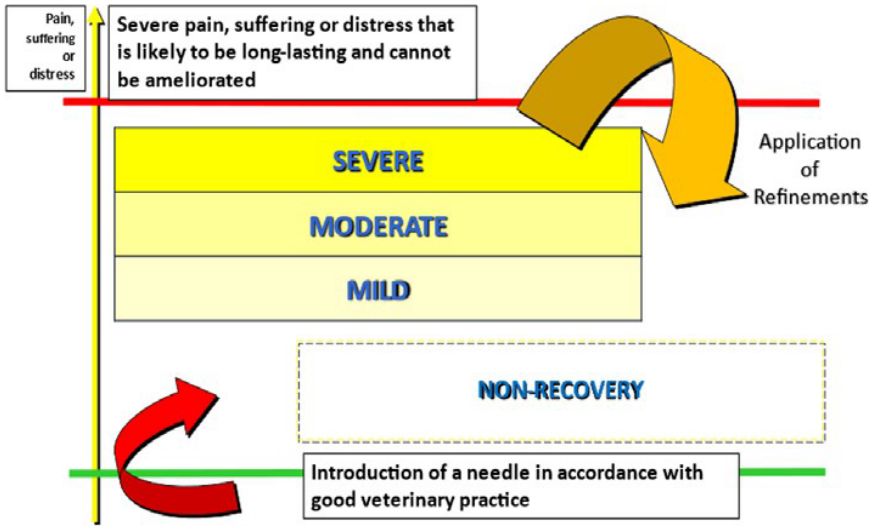
In each case, consideration needs to be given to the expected effects on the animal to determine whether or not there are any steps in a procedure considered to be above the threshold, in which case project authorisation would be necessary.

It is also necessary to consider the cumulative effects of multiple interventions (invasive or non-invasive) that, when applied individually, may not be considered above threshold. For example, single versus multiple short periods of food withdrawal.

1.3.5. Severity classification

For more information, see **Section 2**.

Figure 1.3. Severity classification



Source: Degryse A–D and Denais–Lalieve D. from SESANE at ANSR (Personal Communication)

If 'above threshold' manipulations are required and included in a project application, each procedure is assessed for overall severity and a classification of mild, moderate, severe or non-recovery is applied. (Non-recovery is where the animal is kept under general anaesthesia throughout the procedure and is not allowed to regain consciousness before being killed.)

At the end of each procedure, the project holder reports on the highest actual severity experienced by each animal during the procedure, based on the daily monitoring during the procedure. This may differ from the severity classification assigned prospectively for the entire group of animals. This information is included in the published annual statistical returns.

There is an 'upper limit' on suffering imposed by the Directive (Article 15 (2): 'Member States shall ensure 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'). This may be 'exceeded' only with agreement at the EU level; to date (2013–2025), this has never been requested.

1.3.6. Genetically altered animals

The Directive also regulates the creation and breeding of genetically altered animals, GAAs: 'any course of action intended, or liable, to result in the birth or hatching of an animal or the creation and maintenance of a genetically modified animal line in any such condition'.

The creation of new lines of GAAs requires project authorisation. All offspring are maintained under project authorisation until the line is stable and a decision is made as to whether or not the newly created line is classed as harmful or non-harmful. Harmful lines will continue to be bred under a project authorisation, but non-harmful lines would no longer need to be maintained under an authorised project.

There are a number of complex issues in relation to the use and reporting of GAAs; detailed guidance is available from the European Commission (European Commission: Directorate-General for Environment, 2022). Animals in science – Implementation', European Commission website.

1.3.7. Support personnel within an establishment

Each establishment is required to have a number of persons responsible for various duties under the legislation. These are listed below, together with their main roles.

a) **Person(s) responsible for overseeing the welfare and care of animals in the establishment**

   This role will often be fulfilled by an experienced animal care person / senior animal technologist, although occasionally a veterinarian may assume this role. There could be one or more such persons in an establishment, but each will have clearly defined areas of responsibility.

   The individual should be able to provide independent advice on the welfare and care of all animals within the establishment to minimise suffering and optimise the welfare of animals being bred, kept for use or used in the establishment.

   The individual is generally actively involved on a daily basis in safeguarding the welfare of animals within the establishment, and will have managerial authority enabling them to establish and, in conjunction with the care staff, maintain high standards of husbandry and care.

   This person should champion a culture of care among both husbandry and scientific staff, and be proactive, working, as appropriate, with the DV to promote the implementation of refinements in animal husbandry, care and use, and contributing actively to the work of the AWB.

b) **Person(s) responsible for ensuring that the staff dealing with animals have access to information specific to the species housed in the establishment**

This person ensures that a range of relevant information is made available to those who need it, and that the information is kept up to date as far as possible.

This includes all manner of information on the species housed and used, relating to a varied range of subjects, such as:

- the species used in the establishment;
- animal care and husbandry;
- animal welfare and the Three Rs;
- EU and national legislation, guidance and local rules/information;
- information on new initiatives, technical and practical advancements and good practice in a relevant field of research and in relation to the species concerned.

c) **Person(s) responsible for the education, competence and continued professional development of staff**

This person will generally be involved in coordinating training and ensuring that supervision, competence assessment and continued professional development are undertaken and recorded, rather than directly delivering training or assessing competence.

This person will support all staff members to ensure that all education and training requirements are identified and delivered. This person will also support personnel to ensure that appropriate supervision is available when necessary, and that competence assessments are carried out and delivered to a suitable standard.

d) **Designated veterinarian**

Each establishment has a DV with expertise in laboratory animal medicine charged with advisory duties in relation to the well-being and treatment of animals. This role, in very few circumstances, may be performed by a suitably qualified expert, if deemed more appropriate than a veterinarian, for example where there is no veterinary expertise available for an unusual or seldom-used species, such as squid or another cephalopod.

The role of the DV extends beyond advice on animal disease or health issues; it is an integral part of the continued improvement of scientific practices, in particular with respect to refinements in model design, clinical monitoring and a culture of care. The DV generally contributes to the work of the AWB, and provides input on, for example, anaesthetic and surgical practices, welfare and severity assessment. In some establishments, the DV also contributes to the education and training programme.

1.4. Animal welfare bodies

Each establishment is required to have an **AWB**. It has a number of important roles, advises all staff on the welfare, care and use of animals and promotes the uptake of the Three Rs within the establishment.

The Directive specifies the minimum composition of the AWB; it is to include the person responsible for overseeing the welfare and care of animals in the establishment, and, in user establishments, a scientific member. The DV will also provide input.

In practice, in particular in larger establishments, the AWB often has a broader membership, with the DV also being a member of the AWB.

The Directive also specifies the minimum tasks for which the AWB is responsible (Article 27):

a) advise the staff dealing with animals on matters related to the welfare of animals, in relation to their acquisition, accommodation, care and use;

b) advise the staff on the application of the requirement of replacement, reduction and refinement, and keep them informed of technical and scientific developments in areas relevant to work/animals in the establishment;

c) establish and review internal operational processes as regards monitoring, reporting and follow-up in relation to the welfare of animals housed or used in the establishment;

d) follow the development and outcome of projects, taking into account the effect on the animals used, and identify and advise as regards elements that further contribute to replacement, reduction and refinement;

e) advise on rehoming schemes, including the appropriate socialisation of the animals to be rehomed.

In addition to the statutory functions, many AWBs carry out other functions within establishments, for example providing support for and oversight of the education and training of staff, helping to ensure that suitable facilities are maintained and upgraded as necessary (e.g. by persuading senior management to release the necessary funds) and promoting consistency within the establishment in the application of the severity framework and reporting of actual severity.

Another key function that has evolved in many establishments since the introduction of AWBs is their central role in the promotion of a culture of care. Simply having animal facilities and resources that meet the requirements of the legislation will not ensure that appropriate animal welfare, care and use practices will follow. All those involved in the care and use of animals should be committed to the principles of the Three Rs and demonstrate a caring and respectful attitude towards the animals bred or used for scientific procedures. Without an appropriate culture of care within an establishment, it is unlikely that welfare and scientific outcomes will be optimised.

Empowered animal care staff and veterinarians are essential to a good culture in which animal care and technical staff are respected and listened to and their roles and work are supported throughout the establishment.

1.5. Animal care responsibilities

For breeding and stock animals, primary responsibility for husbandry and care practices lies with the animal care staff. Within an establishment, the person responsible for overseeing the welfare and care of animals and the person responsible for ensuring compliance within the establishment also have responsibilities for ensuring the welfare and care of breeding and stock animals.

For animals undergoing procedures within the framework of a project, the individual carrying out a procedure has primary responsibility for the welfare and care of the animal(s) undergoing that

procedure. The holder of the project authorisation is responsible for ensuring that those working on the project provide suitable monitoring, comply with requirements for minimising animal suffering and ensure that humane endpoints are applied when necessary. Within an establishment, the person responsible for ensuring compliance is also responsible for ensuring that any suffering is minimised; in practice, they often delegate such animal monitoring to care staff and the DV.

The DV is available to provide advice on all aspects of animal health or welfare issues for breeding, stock and animals undergoing procedures and will generally be consulted should problems arise.

For animals undergoing procedures, the project will set out, for each procedure, the adverse effects that are expected, the actions to be taken to minimise any harms and the humane end points defined (i.e. the point at which the animal must be either removed from the study and treated or killed). The DV will generally contribute to discussions on welfare assessment and monitoring, and to the interventions to be made should problems arise.

Occasionally, animals undergoing procedures may be become ill due to, for example, an infection in the animal unit unrelated to the procedures. The scientist and veterinarian need to consider the impact of the disease on the scientific outcomes before determining whether or not the animal should be treated, withdrawn from the study or killed.

Death as an endpoint is to be avoided as far as possible; in the few circumstances where this is not possible, the duration and intensity of suffering must be reduced as much as possible.

Good communication is essential among all those involved in animal care and use, to ensure that animal welfare and care practices are implemented effectively, to prevent avoidable animal suffering.

1.6. Fate of animals

At the end of procedures, animals may be kept alive, rehomed or killed.

In the vast majority of circumstances, animals are killed, as tissues are often required for analysis to complete the scientific studies.

Animals must be killed using an appropriate method that causes minimum pain, suffering and distress. Personnel killing animals must be trained and competent. A list of approved methods is included in the Directive as Annex IV. This includes the commonly used methods for the main laboratory animal species.

Individual Member States can authorise additional methods provided there is scientific evidence that the methods are at least as humane as those in the Directive Annex.

Killing by a method in Annex IV is not a procedure and no project authority is required. However, if a suitable scientific justification is provided in a project application, a different method may be authorised.

1.7. Other related legislation

When using, breeding or supplying animals, in addition to the requirements of the primary legislation (Directive 2010/63/EU), the requirements of other legislation often need to be considered, dependent on, for example, the type of research and the species of animal being used.

The list below is not exhaustive, but contains the most relevant legislation encountered when working with animals in research.

As indicated earlier in this chapter, European directives are incorporated into national legislation. As a consequence, there may be differences among Member States in how the directive's objectives are achieved through national law.

By contrast, EU regulations have direct effect and will have the same detailed requirements in all Member States.

1.7.1. Transport

Transport legislation in Regulation (EC) 1/2005 regulates the transport of live vertebrate animals between Member States and provides for checks on animals entering or leaving the EU. The detailed rules aim at safeguarding animal welfare and preventing injury to or unnecessary suffering for the animals. The legislation is being revised at the time of writing this manual (September 2026). The reader is advised to check the legislation in force. Information on international air transport can be found in the International Air Transport Association (IATA) guidelines (https://www.iata.org/en/programs/cargo/live-animals/).

1.7.2. Animal health

Regulation (EU) 2016/429 on transmissible animal diseases aims to prevent and control animal diseases that can be transmitted to other animals or humans. It sets out requirements for disease prevention and preparation for possible outbreaks (e.g. biosecurity measures), such as the use of diagnostic tools, vaccination and medical treatments; the entry of animals and animal products into the EU and their movement within the EU; and disease control and eradication, including emergency measures such as restrictions on the movement of animals, culling and vaccination.

1.7.3. Veterinary legislation

This will vary among Member States, but, in general, it will set out the role of veterinary surgeons and what can be performed within the scope of veterinary practice, for example in terms of diagnosis, treatment and prevention of animal diseases by use of medicines, vaccines, etc.

1.7.4. Medicines legislation

In the context of animals kept for and used in scientific procedures, there are controls over the acquisition, storage, use and disposal of medicinal products, including substances such as antibiotics, anaesthetics and analgesics (pain relievers). When such medicines are used to treat animals, these will be the responsibility of the DV. When such medicines are used as part of a research programme, the medicines may be obtained for specific research purposes, but are still subject to rigorous control over storage, use and disposal.

1.7.5. Genetic modification legislation

There is legislation controlling the deliberate release of genetically modified plants or animals into the environment (Directive 2001/18/EC). In establishments creating, breeding or using GAAs (covered by the regulations on genetic modification), it is necessary to ensure compliance with the relevant containment, disposal and record-keeping requirements.

1.7.6. Convention on International Trade in Endangered Species of Wild Fauna and Flora

The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) is an international agreement between governments that came into force in 1975.

The convention aims to ensure that international trade in wild animals and plants does not threaten their survival, and it stipulates that all imports, exports and re-exports of species covered by the convention have to be authorised through a system of permits and certificates.

In terms of animal research, CITES legislation (as embodied in Council Regulation (EC) 338/97) has to be considered when planning research on endangered species; the necessary justification must be included in the authorised project, and permits must be secured before animals are sourced.

1.7.7. Wildlife legislation

In many Member States, protection is afforded to wildlife in national legislation, providing protection to, for example, wild birds and their eggs and nests.

If scientists intend either to take animals from the wild or to carry out procedures on animals in the wild, it is important that they comply with any national legislation and have secured any necessary permissions before the work begins.

1.7.8. Human safety and health

Laboratory animal facilities may present risks to the safety and health of people working in them. Employers are required to have health and safety policies in place to ensure that risks are minimised.

The EU framework directive on safety and health at work (Framework Directive 89/391/EEC), adopted in 1989, guarantees minimum safety and health requirements throughout the EU. Member States are allowed to maintain or establish more stringent measures.

References

Animal Technology and Welfare, https://www.atwjournal.com/.

Council Directive 89/391/EEC of 12 June 1989 on the introduction of measures to encourage improvements in the safety and health of workers at work, OJ L 183, 29.6.1989, p. 1, ELI: https://eur-lex.europa.eu/eli/dir/1989/391/oj.

Council regulation (EC) No 33/97 on the protection of wild fauna and flora by regulating the trade therein http://data.europa.eu/eli/reg/1997/338/oj

Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC – Commission Declaration, OJ L 106, 17.4.2001, p. 1, ELI: https://eur-lex.europa.eu/eli/dir/2001/18/oj.

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, OJ L 276, 20.10.2010, p. 33, ELI: https://eur-lex.europa.eu/eli/dir/2010/63/oj/eng.

European Commission, 'ALURES (Animal use reporting – EU system) – EU statistical database on the use of animals for scientific purposes under Directive 2010/63/EU', Section 1: Numbers and origins of animals in research and testing https://webgate.ec.europa.eu/envdataportal/content/alures/section1_number-of-animals.html.

European Commission, 'Animals in science – Implementation', European Commission website, https://environment.ec.europa.eu/topics/chemicals/animals-science_en#implementation.

European Commission: Directorate-General for Environment, Framework for the genetically altered animals under Directive 2010/63/EU on the protection of animals used for scientific purposes, Publications Office of the European Union, 2022, https://data.europa.eu/doi/10.2779/499108.

IATA, 'Live animals', IATA website, https://www.iata.org/en/programs/cargo/live-animals/

Regulations (EC) 1/2005 on the protection of animals during transport and related operations. http://data.europa.eu/eli/reg/2005/i/ei.

Regulations (EU) 2016/429 on transmissible animal diseases. http://data.europa.eu/eli/reg/2016/429/oj.

Further reading

Education and Training Platform for Laboratory Animal Science (ETPLAS) website, https://etplas.eu/en.

Federation of European Laboratory Animal Science Associations (FELASA) website, https://felasa.eu/.

Guillén, J., Prins, J-B., Smith, D., Degryse, A.-D. (2014), 'The European framework on research animal welfare regulations and guidelines', in: Guillén, J. (ed.), *Laboratory Animals*: Regulations and recommendations for global collaborative research, Academic Press, San Diego, CA, pp. 117–188.

Russell, W. M. S. and Burch, R. L. (1959; reprinted 1992), The Principles of Humane Experimental Technique, Universities Federation for Animal Welfare (UFAW), Wheathampstead.

Self-assessment questions

1. What is the primary legislation in Europe that authorises the use of animals for research purposes?
2. What animals are included within the scope of the Directive?
3. Which personnel are required within each research establishment to safeguard animal welfare?
4. Explain the role of the AWB.
5. What other legislation needs to be considered when animals are used in research?

Additional terminology

Competent authority. The government has the overall responsibility for meeting legal obligations within the Directive. Although, in many Member States, all the obligations are met within one department, in others, tasks are designated to (a) competent authority/ies responsible for specific individual roles. These roles, which must be carried out by a competent authority, are the authorisation of establishments, inspections, the evaluation of projects, the authorisation of projects and the assessment of projects on their completion (retrospective assessment of projects).

The Three Rs. The Directive embeds within the legal framework the principles of the Three Rs (Replacement, Reduction and Refinement), which were first described by Russell and Burch in 1959:

— **Replacement** can be defined as methods, strategies or approaches that do not involve the use of live animals;

— **Reduction** covers any approach that will result in fewer animals being used to achieve the same objective;

— **Refinement** signifies the modification of any procedures or practices from the time the experimental animal is born until its death to minimise its suffering and enhance its well-being, or by moving from species that are considered more sentient to those considered less sentient.

The application of the Three Rs is underpinned within the training requirements for scientists and care staff and is subject to continuous review throughout the duration of a project (which can be authorised for up to five years), such that scientists and care staff are required to introduce new knowledge/developments (e.g. improved anaesthesia or analgesic regimen) during the life cycle of a project.

The Three Rs must also be respected in breeding and supplying establishments, for example refinement through improved care and accommodation practices, reduction of numbers of surplus animals through improved stock management, and moving from invasive to non-invasive genotyping methods in the breeding of GAAs.

Use. The 'use' of an animal within a project extends from the time the procedure (or the first step in a series of steps (multi-step)) is applied to the animal to the time when the observations are completed, or the collection of data (or other products) for a particular scientific purpose (usually a single experiment or test) is completed. At the end of the use, the animal may be killed or kept alive, provided it is experiencing only mild adverse effects and continues to receive appropriate care and accommodation.

Reuse. If the appropriate permission has been granted for a project, it is possible to use the same animal in an additional procedure. If there is no scientific need to use the same animal again, any further use of that animal is reuse. Permission for reuse is subject to consideration of the effects of the previous procedure(s) and in accordance with veterinary advice.

National Committees. Each Member State has established a National Committee for the protection of animals used for scientific purposes. Its functions are to advise competent authorities and AWBs on issues relating to the acquisition, breeding, supply and use of animals, and to promote sharing of best practice.

Many National Committees have published guidance on aspects of the legal framework, for example, education and training, advice to avoid duplication of procedures and re-homing. National Committees also host meetings of AWBs to promote consistency and share good practice.

There are also network meetings and exchanges within the EU for National Committees and AWBs.

Non-human primates. The EU legislation imposes significant constraints on the use of non-human primates, with additional limitations on endangered species. Furthermore, great apes cannot be used, except in exceptional circumstances subject to approval at the EU level. During the first 15 years of the Directive (2010–2025), no requests to use great apes were made.

2. Application of the severity assessment framework

This chapter provides you with an overview of the severity assessment framework and its contribution to enhancing transparency, improving animal welfare and promoting implementation of the Three Rs. It provides information on the nature of regulated procedures, the classification of procedures, why severity assessment is a key component of the regulation of animal use in research and the reporting of actual severity (module 12).

2.1. General introduction

Directive 2010/63/EU on the protection of animals used for scientific purposes requires that any pain, suffering or distress is minimised as far as possible.

It is recommended that Chapter 1 on the legal framework within the EU is read in advance of this chapter, as much of the terminology used within the Severity Assessment Framework is explained there.

This chapter provides an overview of the Severity Assessment Framework within the Directive. This includes the measures that have to be taken during the development, performance and review of projects using live animals in research, and how these contribute to improving animal welfare, promoting the implementation of the Three Rs and enhancing transparency for the general public on the use of animals in research.

The framework of the Directive requires that, during the design of a project, consideration is given to the likely adverse effects on the animals and the measures to be taken to minimise these, and that the **Three Rs** are fully implemented. A prospective severity classification for each procedure has to be authorised in the project.

During the course of all procedures, the effects on the animals need to be carefully monitored and recorded, and any further opportunities for refinement applied. At the end of a procedure, the actual severity experienced by each animal needs to be reported. On completion of certain projects, for example those involving non-human primates or severe procedures, there needs to be a retrospective assessment of the entire project.

Consideration of the effects of procedures at all stages of a project provides a firm base for minimising the negative welfare consequences for the animals.

Effective severity assessment will promote improved scientific data quality (due to better welfare), and will improve communication among those responsible for using, caring for and monitoring animals.

Consistency is important in the assessment and reporting of severity, for animal welfare, for the scientific research community (improved science and a level playing field across the EU) and to maintain the confidence of the general public.

Achieving the necessary consistency requires well-trained personnel, accurate monitoring, and good cooperation and understanding among all those involved in the care and use of the animals.

2.2. Project and procedures

Scientific procedures are contained and authorised within a project. The project application includes the scientific objectives and potential benefits of the work, and details of the procedures to be carried out on the animals.

2.2.1. Project

Before being authorised by the relevant competent authority within a Member State, the project is evaluated to determine whether the harms to the animals are outweighed by the potential benefits the project will bring.

The details of the scientific procedures give estimates of the number of the animals to be used, the potential harms and measures to be taken to minimise these. Finally, a severity classification is assigned to each procedure.

2.2.2. Procedure

A procedure is one (single-step procedure) or more (multi-step procedure) technical acts carried out on an animal for an experimental or other scientific purpose, which may cause that animal pain, suffering, distress or lasting harm.

2.3. Thresholds of severity

2.3.1. Lower threshold

A procedure is defined in the Directive as any use of an animal for an experimental or other scientific purpose that may cause the animal a level of 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.

This definition sets the **lower threshold** for a procedure, that is, if what you are intending to do to the animal does not cause the same or a higher level of pain, suffering, distress or lasting harm than the introduction of a needle in accordance with good veterinary practice, then the action is not considered a procedure and no project authorisation is necessary. Consideration needs to be given to both invasive (e.g. blood sampling) and non-invasive (e.g. restraint or single housing) manipulations when deciding the likelihood of it reaching the minimum threshold of pain, suffering, distress or lasting harm.

In simple terms, if injections or blood samplings are required, these constitute procedures. However, other interventions need to be considered on a case-by-case basis to determine whether pain, suffering, distress or lasting harm may be caused.

For example, simple observational studies with no interventions in normal husbandry and care practices would not cause any adverse effects and therefore would be considered below the lower threshold.

Similarly, if an assessment of changes to husbandry and care practices is proposed, such as the addition of a novel enrichment device, which can be reasonably expected to improve welfare, then this study may not be considered a procedure, and no project authorisation would be necessary. However, such a study should still be well designed, such that any changes can be properly assessed and, where appropriate, reported/published.

Other examples of 'below threshold' procedures are the assessment of minor adjustments to diet that are not expected to have any adverse effects, adding inert markers to the diet to follow the passage of material through the digestive tract, a short period of food withdrawal (e.g. up to 24 hours for an adult rat, keeping in mind that the duration will differ depending on the species and life stage, thus requiring a case-by-case determination) and simple behavioural tests (e.g. open field testing).

Although these would be deemed below the minimum threshold, it is also important to consider how frequently these would be used on animals in the study, and whether or not they are used in combination. There may be a **cumulative effect** that, as a consequence, may shift the study from 'below' to 'above' the threshold, at which point project authorisation would be necessary.

2.3.2. Upper limit

The Directive states that a procedure must not be performed if it involves severe pain, suffering or distress that is likely to be long-lasting and cannot be ameliorated.

Note. Although there is a provision in the Directive that could permit such a procedure in very exceptional circumstances, subject to approval at the EU level, no such procedure has been requested since the Directive took effect in 2013.

2.4. Assignment of severities

The legal framework requires that all procedures are classified prospectively within the project, and that the actual severity experienced by each animal during the procedure is recorded and reported.

This framework is very important, as it provides opportunities at all stages of a project (during the design phase and the study phase and following completion) to consider the potential and actual adverse effects, take appropriate action and assess the effectiveness of such action to minimise pain, suffering and distress. Moreover, it provides a practical, jointly agreed and consistent tool on how best to monitor the animals to ensure their welfare.

2.4.1. Prospective severity classification

Each procedure in a project is assigned a prospective severity classification. A procedure can be classified as 'non-recovery', 'mild', 'moderate' or 'severe' on a case-by-case basis.

Non-recovery

Procedures that are performed entirely under general anaesthesia from which the animal will not recover consciousness are classified as 'non-recovery'. No manipulation (e.g. dosing or sampling) of the animal is carried out before the administration of the general anaesthesia.

Note. Non-recovery should not be confused with a procedure that has multiple steps (without general anaesthesia) and in which the final step is under general anaesthesia, after which the animal is killed without recovery. Such procedures should be classified under one of the following classifications based on the likely impacts of the steps preceding the final step under general anaesthesia.

Mild

Procedures on animals that are likely to cause the animals short-term mild pain, suffering or distress, and procedures with no significant impairment of the well-being or general condition of the animals, are classified as 'mild'.

Moderate

Procedures on animals that are likely to cause the animals short-term moderate pain, suffering or distress, or long-lasting mild pain, suffering or distress, and procedures that are likely to cause moderate impairment of the well-being or general condition of the animals, are classified as 'moderate'.

Severe

Procedures on animals that are likely to cause the animals severe pain, suffering or distress, or long-lasting moderate pain, suffering or distress, and procedures that are likely to cause severe impairment of the well-being or general condition of the animals, are classified as 'severe'.

When determining an appropriate prospective severity classification, it is necessary to consider the impact on the animal of each step of the planned procedure (**Figure 2.1**).

— *What is being done to the animals?*

— *What effect will this have on the animals?*

— *How much suffering might it cause?*

— *What interventions can be included to reduce the impact on the animals?*

When assigning a procedure to a particular category, the type of procedure and a number of other factors will be taken into account. All these factors will be considered on a case-by-case basis.

The factors related to the procedure include:

— the type of manipulation;

— the nature of the pain, suffering, distress or lasting harm caused by (all steps in) the procedure – consider the intensity, duration, frequency and complexity;

— cumulative suffering within the procedure;

— impact on the animal's ability to express natural behaviour;

— consideration of non-invasive factors such as any restrictions on housing, husbandry and care standards, for example single housing or restriction in a small metabolism cage.

The severity classification can also be influenced by the species, strain and stage of development, and habituation to (or training for) the planned procedures.

The severity classification for a procedure should be set at the highest level that an individual animal may experience within the procedure, that is, the worst-case possibility, and it must be set at the highest level expected at any stage of the procedure.

For example, in a group of 10 animals, only one animal is expected to exhibit moderate clinical signs following injection with a test substance, with the remaining animals not expected to exhibit any adverse effects. The prospective severity classification for that procedure would be **moderate**.

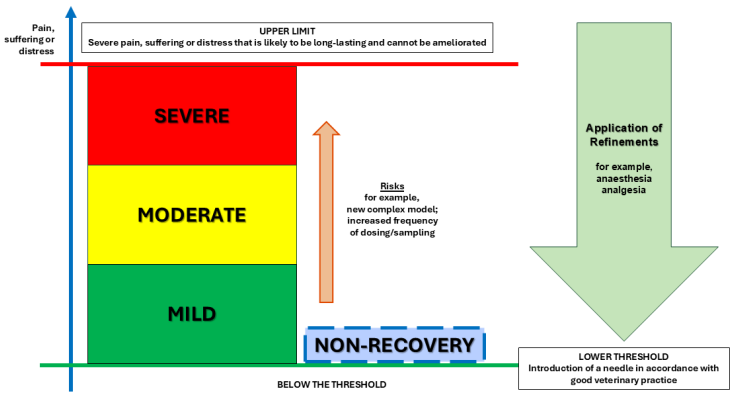
In another example, animals may undergo a surgical procedure, for example surgical implantation of a telemetry blood pressure monitoring device. Following recovery from the surgery, the animals will have their blood pressure monitored in their home cage for several months. In this case, the surgery, even with appropriate analgesia (pain relief), would be classified as **moderate**. Although the animals

would remain on the study for months without adverse effects, the highest severity expected to be experienced was **moderate**, so this would be the appropriate prospective classification.

Annex VIII of the Directive contains further examples of typical procedures under different categories, which can be used as a **starting point** before considering other factors that may affect the final severity classification.

2.4.2. Assessment of actual severity

Figure 2.1. Terminology and prospective classification of severity



Source: Anderson D. and Louhimies S.

Assessment and recording of actual, experienced severity during the course of the procedure

During the design phase of the project, it is important that an appropriate monitoring system is agreed such that all those involved, including scientists, care staff and veterinarians, are fully aware of which parameters will be monitored (e.g. body condition, food/water intake).

The details of the procedure will be included in the project plan, and the assessment/monitoring system should be tailored to ensure that all relevant elements are included.

The frequency of assessment and recording will depend on the procedure and may vary over the course of the project. For example, there may be hourly monitoring immediately after a surgical procedure, but, following recovery, twice-daily assessment may suffice.

The recording system should be clear and the terminology used understood by all. It should include welfare indicators that are readily and reliably recognised, are good measures of welfare, are relevant to the particular study and are practical to carry out with minimum disturbance to the animals.

The signs commonly recorded include overall appearance (coat; body condition; any discharge from, for example, eyes or nose), behaviour, respiration, food/water intake, environment (e.g. diarrhoea, wet bedding).

Study-specific indicators may also be included, for example using calipers when measuring subcutaneous tumour growth in a cancer study.

When adverse effects are identified, it is important that, whenever possible, action is taken to minimise them. Many will have been identified clearly in the project plan; for example, pain relief (analgesia) will be routinely provided before and after a surgical procedure, and will be continued if

clinical signs of pain remain. Similarly, husbandry practices may need to be adjusted; for example, in diabetic animals, more frequent changing of bedding will be necessary.

The procedure will also define humane endpoints for the study; when these are reached, to eliminate further suffering, animals must either be removed from the study and treated or killed. Death as an endpoint must be avoided whenever possible, and is permitted within a project authorisation only with very good justification.

If the severity classification of the procedure has been or is likely to be exceeded, then discussion is necessary with the project authorisation holder to determine whether the project needs to be amended.

Reporting of actual severity

At the end of a procedure, the actual severity experienced by each animal has to be agreed and reported for inclusion in the annual statistical reports on animal use.

As the care staff, veterinarians and scientists are all generally involved in the care of the animals, their input is important in the review of the animals' clinical/welfare records.

The actual severity to be reported is the highest level of severity experienced by an animal at any stage in the procedure. The cumulative effects also need to be considered; that is, having considered the elements that may increase severity, does any cumulative effect move the severity up to the next level?

The reporting of severity should reflect only the effects of the scientific procedures, not unrelated effects such as an outbreak of disease or injury due to aggressive behaviour / fighting. Note that these unrelated effects still need to be dealt with, and steps should be taken to avoid recurrence.

Cumulative severity can be estimated from the nature and duration of adverse and unexpected effects encountered during the course of the procedure, and the effectiveness of actions taken to mitigate these effects. For example, training to collaborate in the procedure or acclimatisation can reduce the negative welfare impacts on the animal.

When considering the lifetime experience of an animal, for example if a request is made to reuse an animal, it is important to consider not only the **direct suffering** caused by the scientific procedures, but also other factors unrelated to the procedure that can have a negative impact on welfare, for example transport, marking for identification or capture in the wild in the case of field studies. These factors contribute to **contingent suffering**.

2.4.3. Consistency in the assessment of severity

It is important that there are systems in place to promote a consistent application of the severity framework.

Without a consistent approach, there are likely to be differences in how severity is classified, monitored, recorded and reported. This can negatively affect animal welfare, the scientific data and the scientific community in its ability to conduct research, and can influence public confidence in the use of animals in research.

Effective severity assessment requires:

- a team approach, with input from people with different expertise, experience and priorities;
- good planning – before the study begins, ensure everyone is aware of the proposed procedures and individual responsibilities;

— education and training of all personnel involved – an EU e-learning module is available on severity assessment (https://etplas.eu/en);

— appropriate, tailored recording systems (e.g. scoring sheets tailored to the procedure), to assist in the identification of welfare issues;

— appropriate protocols for assessing behaviour and clinical signs;

— use of the observations to make an informed judgement on the nature and level of suffering;

— awareness of the severity of each procedure and what action to take if this is reached or exceeded;

— a consistent approach to overall judgements on actual suffering (mild, moderate, severe) for reporting of actual severity.

Promoting consistency

To promote consistency, ensure that there is:

— expertise on animal health, welfare and behaviour;

— good communication among all those responsible for conducting the study and monitoring the animals (top-down and bottom-up, between researchers, care staff and veterinarians);

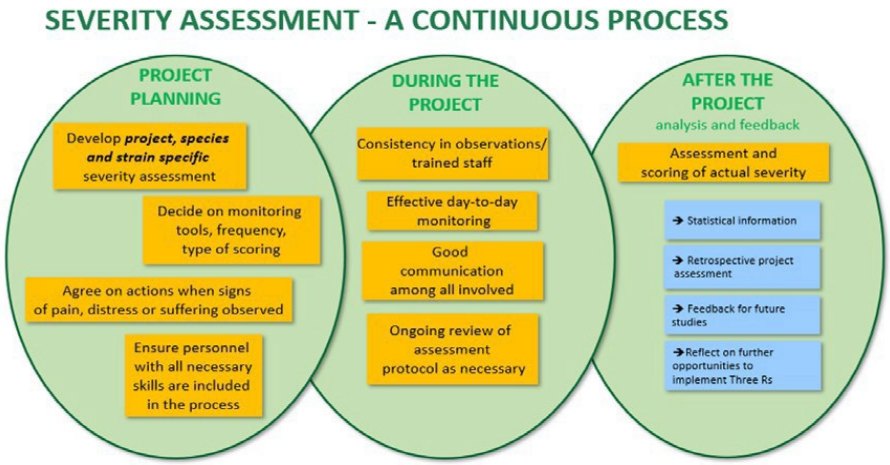
— oversight, that is, regular review of outcomes within the research group and establishment (e.g. the AWB), with other establishments and with other groups using similar animal models.

2.5. Summary

Figure 2.2 demonstrates the importance of the severity framework as a continuous process that begins at project inception and continues until the outcomes of the project are reported.

To be successful, it is crucial to have input from, and good communication between, the scientific and care staff.

Figure 2.2. Severity assessment: a continuous process



Source: Severity Assessment Framework (European Union,2018)

References

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, OJ L 276, 20.10.2010, p. 33, ELI: https://eur-lex.europa.eu/eli/dir/2010/63/oj/eng.

Education and Training Platform for Laboratory Animal Science (ETPLAS) website, https://etplas.eu/en.

Further reading

European Commission, 'ALURES (Animal use reporting – EU system) – EU statistical database on the use of animals for scientific purposes under Directive 2010/63/EU', Section 2: Uses in research and testing https://webgate.ec.europa.eu/envdataportal/content/alures/section2_number-of-uses.html.

European Commission, 'Animals in science – Implementation', European Commission website, https://environment.ec.europa.eu/topics/chemicals/animals-science_en#implementation.

Hawkins, P., Morton, D. B., Burman, O., Dennison, N., Honess, P., *et al.* (2011), 'A guide to defining and implementing protocols for the welfare assessment of laboratory animals: eleventh report of the BVAAWF/FRAME/RSPCA/ UFAW Joint Working Group on Refinement', *Laboratory Animals*, Vol. 45, pp. 1–13.

Russell, W. M. S. and Burch, R. L. (1959; reprinted 1992), The Principles of Humane Experimental Technique, Universities Federation for Animal Welfare (UFAW), Wheathampstead.

Smith, D., Anderson, D., Degryse, A. D., Bol, C., Criado, A., *et al.* (2018), 'Classification and reporting of severity experienced by animals used in scientific procedures: FELASA/ECLAM/ESLAV Working Group report', Laboratory Animals, Vol. 52, pp. 5–57.

Contribution of this chapter to the Three Rs

— There is a legal requirement to refine the use of animals in scientific procedures, whenever possible.

— Minimising pain, suffering and distress is the responsibility of everyone working with live animals.

— Good planning and communication on agreed intervention points and regular monitoring allow timely action to be taken when unnecessary animal pain, suffering or distress is noted.

— Death as an end point should be avoided.

Self-assessment questions

1. What is the lower threshold set in the legislation above which project authorisation is required?
2. Give an example of a single-step procedure and of a multi-step procedure.
3. List the categories of severity that can be assigned to a procedure.
4. What is the difference between a prospective severity classification and the reporting of actual severity?
5. Why is a consistent approach to severity assessment important?

3. Ethics, the Three Rs and the culture of care

After reading this chapter, you should understand the ethical and welfare issues that are raised by the use of animals in scientific procedures generally and in your own place of work. You should understand the principles of the Three Rs and the culture of care, and how you can promote them.

You should also know where further information can be obtained to keep up with new advances in animal care and welfare (learning outcomes in module 2).

3.1. Introduction

This chapter discusses the welfare and ethical aspects of the use of animals for scientific procedures. The public's attitude to the scientific use of animals in scientific research is considered, followed by the responsibilities of all the people who work in animal research facilities and, finally, how animal welfare can be maintained and improved. Fundamental to the animal welfare and ethical aspects of the use of live animals in science are the principles of humane experimental technique (the Three Rs) and the culture of care. The chapter begins by outlining the meaning of ethics.

3.2. Ethics

Ethics is a branch of philosophy that deals with the concepts involved in practical reasoning, answering questions like what is right? what is wrong? duty etc. (Blackburn, 2016). Applied ethics is a branch of the subject that uses these concepts to make decisions in the real world, for example whether or not animals should be used in science and, if so, how. Philosophers through the ages have set out to identify principles to help find reasonable answers to these and similar difficult questions. Analysis of these theories is outside the scope of this book, but works by Dolan (1999, 2007) are recommended for those who are interested in the subject and wish to consider ethics in relation to the use of animals in science in more depth. One theory is worth outlining because it is often used in connection with the use of animals in science: utilitarianism. In basic terms, it advocates examining all possible outcomes from all the possible actions that can be taken in specific circumstances and choosing the one with most utility or desirability. A decision arrived at by utilitarian reasoning is sometimes referred to as the one that brings the maximum happiness to the maximum number, although that is a very simplistic interpretation. Theories such as utilitarianism are tools: they help people to make decisions, but they do not guarantee that a 'correct' decision will be made, and two individuals using the same tool may come to different conclusions.

In circumstances where human or animal well-being is concerned, a set of ethical principles on how they should be treated emerges and a code of ethics is formulated. All people working in the field are expected to comply with the code and, because the potential to cause harm is high, the code is enforced. Enforcement could be by professional bodies that have the power to remove the right to practise of individuals who breach the code, or it could be by law; sometimes it is by both. In the case of animal procedures in the EU, enforcement is through Directive 2010/63/EU.

3.3. Public attitude to the use of animals in science

The latest published statistics on the use of animals in scientific procedures can be seen in **Chapter 1.2**. Although a number of significant advances have been made in discovering alternatives to animal use in science, and the aim is to find ways to replace all animal use, currently animal use is still

considered necessary. The involvement of animals in research continues to contribute to major advances in biomedical knowledge, and to ensuring that new drugs are safe to use.

Clearly, the advances made from the use of animals in biomedical research are of benefit to the public. However, not all of the public agree that they should be used, and many of those who do agree offer only qualified support. A Eurobarometer survey on science and animals showed that 66% agreed with scientists using animals, provided they were the smaller species, such as mice, and the research led to improvement in health and well-being. Only 44% thought that dogs, non-human primates and other large animals should be used (European Commission, 2010). These opinions are reflected in a 2016 Ipsos poll (Clemence and Leaming 2016) on the attitudes to animal research in the United Kingdom: 65% of respondents accepted the need to use living animals in science as long as it was for medical research and there was no alternative. A slightly higher figure of 71% accepted the use of animals in scientific research as long as there is no unnecessary suffering and there is no alternative. Many people surveyed did not feel well informed, and this is supported by the fact that 35% of respondents thought that cosmetic product testing was allowed, when it has been banned in the United Kingdom since 1998 and in the EU since 2013.

All opinions that emerge from these polls must be taken seriously; they are honestly held and should be respected. Within society, opinions vary and include the following.

— Some are violently opposed to the use of animals in scientific research and believe the results of animal experiments have no relevance to the human condition.

— Others hold similar views but without the violence.

— Many, including some of those who work with research animals, would be in a group that, while unhappy with the use of animals in scientific research, accept that it is necessary and does produce valuable results. They see their aim as improving the conditions and standards of care of animals while working to find alternatives to their use and thus eliminate the need to use animals completely.

This is what Russell and Burch were proposing in their book The Principles of Humane Experimental Technique, first published in 1959.

3.4. Humane experimental technique: The Three Rs

The principles of Replacement, Reduction and Refinement (The Three Rs) were first proposed in the 1950s by William Russell and Rex Burch, research fellows working with the Universities Federation for Animal Welfare (UFAW). There was concern that animals were not being treated as well as they should be when being used for scientific research. Russell and Burch were asked to investigate and write a report on their findings. Their report was published as The Principles of Humane Experimental Technique (1959). They proposed, for the first time, that scientists consider other, non-sentient, ways of achieving their aims, rather than automatically selecting animals. In other words, they should look for ways to **replace** animals in their experiments. It was Burch who coined the word 'alternatives' for non-sentient experimental models.

Their investigation found that more animals were used in experiments than were necessary, so they proposed that the numbers should be **reduced** to the minimal numbers that would produce statistically valid experimental results.

The authors also proposed that experiments should be **refined**, by which they meant that any harmful effects the animals experienced during the experiment should be minimised.

An important part of Russell and Burch's humane experimental technique was that, not only should their proposals be put in place, but also the proposals should be improved and extended. For instance:

— existing non-sentient experimental models should be used, and new alternatives should be developed;

— the numbers of animals used in experiments should be reduced, and new ways should be considered to make further reduction possible;

— new ways of refining experiments should be developed and extended to include all aspects of the life of an animal in a research facility.

These recommendations grew into what became known as the principles of the Three Rs.

Concern for animal welfare was not confined to laboratory animals. Ruth Harrison published a book called Animal Machines (Harrison, 1964), which drew attention to the appalling conditions suffered by intensively farmed animals. The UK government set up a committee, chaired by Professor Roger Brambell, to investigate the welfare of intensively farmed animals. The committee reported in 1965 and, as part of the report (Brambell, 1965), it proposed what have become known as the Five Freedoms of animal welfare:

1. **freedom from hunger or thirst** by ready access to fresh water and a diet to maintain full health and vigour;
2. **freedom from discomfort** by providing an appropriate environment, including shelter and a comfortable resting area;
3. **freedom from pain, injury or disease** by prevention or rapid diagnosis and treatment;
4. **freedom to express (most) normal behaviour** by providing sufficient space, proper facilities and company of the animal's own kind;
5. **freedom from fear and distress** by ensuring conditions and treatment that avoid mental suffering.

These freedoms have subsequently been adopted as the basis of animal welfare codes by many organisations around the world.

Although the Brambell report had a fairly rapid impact, it was not the same for Russell and Burch's book, which took nearly 20 years before its importance was noticed and acted upon. It then became a major influence on the development of the law protecting laboratory animals around the world and is now central to the conduct of experiments using animals.

The United Kingdom enacted the first law to protect experimental animals, the Cruelty to Animals Act of 1876. This was replaced by the Animals (Scientific Procedures) Act of 1986, in the same year that the EU (at that time the European Economic Community) adopted its first Directive for the protection of animals in science. Both these pieces of legislation were based on the principles proposed by Russell and Burch. The EU Directive was updated in 2010 and is unique in the world because it sets as its final goal the full replacement of all animals used for scientific and educational purposes, and the EU is taking concrete action towards that goal (https://environment.ec.europa.eu/topics/chemicals/animals-science_en).

3.4.1. Examples of replacements

The techniques used to replace live animals in experiments include the use of freshly harvested tissues and organs, tissue culture cell lines, invertebrate organisms, computer programs and humans.

Tissue culture

Methods using tissues are called *in vitro* (in-glass) methods whereas those using whole living animals are called *in vivo* (in life). To obtain animal tissues, often an animal has to be killed, what makes this a replacement method is that, at the time the experiment is done, the animal cannot feel anything because it is dead, that is, it is non-sentient. Techniques that do not use sentient animals are referred to as relative replacement techniques, whereas those that do not use animals at all are referred to as absolute replacement techniques. Tissue taken from an animal are able to be used for many experiments; some cell lines have been in existence for many years. Tissues are often harvested from animals killed for other reasons, for instance the corneas of cattle killed for food are removed and used for some of the tests for chemical irritancy, and tissue can be taken from animals killed at the end of other experiments.

Recent advances in alternatives technology include body-on-chip devices (also called body-on-a-chip devices) and organ-on-chip devices, whereby interactions between tissues, organs and tissue fluids can be simulated. They have the potential to drastically reduce the numbers of animals needed for toxicology testing. See BioTechniques (2023).

Computers

Computers have a role in replacing animals in experiments. Databases of journals make searching the scientific literature very quick and it is possible to find that the intended experiment has already been done elsewhere. Computers can be used to predict the probable effects new drugs will have, based on the knowledge gained from studies on similar molecules. Computers can help to plan experiments and to collect and analyse data. Artificial Intelligence (AI) has been predicted to be able to greatly reduce the number of animals used in experiments. One example is to link AI to one or more of the body-on-chip devices to enable more information to be gathered and processed.

Humans

Humans have long been used in experimental trials, and there are many advantages to using them in biomedical experiments. Humans are the main beneficiaries of biomedical experiments (including drug development), so it means that results are directly applicable to a wider population. However, there is a limit on the type of test that is socially acceptable and for which people would volunteer. The number that can be used in any experiment is small, all experiments have to comply with strict ethical standards and if anything goes wrong there may be criminal proceedings against the scientists running the experiments. Trials involving human subjects are performed all the time, but only when other tests, often involving animals, have indicated the level of risk.

New technologies, such as body-on-chip devices, will involve humans as donors of tissues and organs.

3.4.2. Examples of reduction

Experimental design

For any experiment, there will be a specific number of animals that must be used to ensure that the results gained are valid. This specific number will depend on the type of animal, the type of experiment

and the conditions in which the experiment is conducted; it will be arrived at by applying statistical principles to the experimental design. Therefore, good statistics-based experimental design is essential if animal lives are not to be wasted. Statistics refers to a number of mathematical formulae that can be applied to experimental results to distinguish between those that are real and those that are due to chance (Festing, 1994).

Control of experimental variables

Many factors, apart from those connected with the experiment itself, can affect the experimental results; in fact anything that can cause a change in the animal's physiology, no matter how minor, may produce abnormal experimental results. These are called external variables. The following are some examples.

- Environmental factors: fluctuations in environmental levels, for instance the environmental temperature, not only affect the comfort of the animal but also can have a major effect on the results of some experiments (**Table 5.3**).
- Diet: changes in the diet an animal eats can also affect some experiments, so it is necessary to make sure that the diet is not changed during an experiment.
- Health status: disease agents cause obvious distress to animals, stimulate the immune system and cause pathological changes, all of which invalidate experimental results. In addition, animals may die due to the combination of the illness and the adverse effects of the experiment. To protect animals and experimental work, all facilities install barriers to prevent the entry of disease agents (see **Chapter 4.3**).
- Genetics: the genetic make-up of an animal can influence its response to an experimental treatment. As an example, there are genetic differences between all the members of an outbred strain of mice (see **Chapter 8.6.2**), so a variation in the results each animal showed would be expected in an otherwise controlled experiment. Each member of an inbred strain of mice (see **Chapter 8.6.1**) is genetically identical to every other member of the strain, so it would be expected that each animal in the strain would produce identical results in a controlled experiment. It follows that the effect of the experiment would be more clearly identified if inbred animals were used, and fewer animals would be needed to obtain a significant result. However, it must be noted that inbred strains are not suitable for all types of experiment.

Other ways in which the number of animals used in animal facilities can be reduced include the following:

- Controlling breeding means that only the number needed for experiments are bred.
- If animals have to be killed (e.g. ex-breeding stock), tissue is harvested and stored for use in replacement techniques.
- Technological advances, such as modern imaging techniques (e.g. magnetic resonance imaging), allow changes that occur over time to be observed in one animal. Without these techniques, batches of animals would have to be killed at stages throughout the study.

3.4.3. Examples of refinement

Refinement is concerned with minimising the pain, suffering, distress and lasting harm an animal may experience during projects, in breeding colonies or in holding rooms. It has been said that animal

care staff have the greatest opportunities to improve animal welfare under the refinement section of the Three Rs.

Examples of refinement include the following.

— Anaesthesia, analgesia and sedation.

  Appropriate use of drugs to control pain and calm animals during procedures is an obvious way to lessen the harmful effects of experimental procedures, but they must be used only by people skilled in their use.

— Training and habituation of animals.

  Animals are far less stressed by situations if they know what to expect. Probably the most obvious example of this is when an initially wary animal becomes calmer and often welcoming of being handled by an experienced and gentle handler. A trusted handler can then get the animal used to any gentle restraint methods that may be necessary if the animal has to undergo procedures (see **Chapter 13.2**). Another example would be exposing an animal to the strange environment of a metabolism cage for short periods before it is placed in one for a longer experiment; they usually find being housed in metabolism cages very stressful, but appear much calmer after several trial periods. This is a welfare advantage, but it also benefits the experimental study (Damon *et al.*, 1986). Larger animals, such as non-human primates, can be trained to present their arms to have blood removed without the need for restraint.

  Training prepares the animals for procedures, minimises the need for restraint and leads to a more successful outcome for the animal and the experiment.

— Training of staff.

  All staff must be competent to carry out the duties allocated to them in scientific establishments. Staff caring for animals must be aware of the signs that could indicate an animal is sick, in pain or in distress. This is most important following a procedure, but is applicable to all animals in their care. Keeping up to date with the latest research in the field is essential because it will improve the chances of identifying problems early; for instance, using face grimaces to gauge the degree of pain that rodents and rabbits are undergoing appears to be a more reliable early indicator than previous methods (see **Chapter 4.5.4**).

  Any person carrying out a procedure on an animal must be aware of the anatomy of the animal, the effect that the procedure will have, the severity classification and the humane endpoint, and be skilled in the procedure itself.

— Choice of species.

  Refinement can also be achieved by moving from species and/or life stages that are considered more sentient to those that are considered less sentient, such as substituting an adult fish with those at earlier life stages (e.g. before they enter under the scope of the Directive) or substituting fish with daphnia. These are both considered methods of refinement, as they are likely to reduce the pain, suffering and distress experienced by the animal; however, they still require the use of live animals.

  Some other typical examples include the use of non-vertebrate organisms such as *Drosophila melanogaster* (fruit fly), which has been used for genetic studies for over a hundred years and is also used in neurobiology studies, and the nematode worm *Caenorhabditis elegans*, which is used for genetic development studies.

Refinement does not apply only to animals undergoing procedures; it covers all animals in a facility. It goes beyond making sure that they are provided with comfortable housing, the correct environment, a suitable diet and gentle handling, and people to inspect them regularly and take action if they are in distress. Care involves ensuring that the psychological needs of the animals are met, as well as the physical ones mentioned above. Environmental enrichment aims to address these needs.

3.4.4. Environmental enrichment

Environmental enrichment aims to improve the daily existence of laboratory animals. It seeks to provide an environment that engages the animal's interest and allows them to fulfil as many of their behavioural needs as possible. Without stimulation, animals become morose and begin to show signs of stress or distress (see **Chapter 4.5.3**).

When considering environmental enrichment, it is not just a matter of putting something in the animal cage/ enclosure in the hope that the animals will use it. Cages and pens are limited spaces and should not be cluttered with 'furniture'; what is added to the enclosure should have the aim of satisfying a particular need of the animal. Animal behaviourists have identified these psychological needs, and they are a good place to start when considering provision of enrichment. These needs include the following.

The need for stability and security

In the wild, animals usually have dens, burrows or treetops to return to for safety when they are in danger, when they need to rest or when they need to get away from their fellow species. In captivity, this need is partially provided by nesting materials that they can hide in, tubes, drainpipes and mouse Des. Res™ (**Figure 3.1** and **Figure 3.2**).

Figure 3.1. Mouse Des Res

Source: LBS Biotech

Figure 3.2. Rabbits in floor pens with tubes and boxes

Source: Martin Heath

Cage/enclosure design is also important; for instance, if a marmoset is alarmed in the wild, it will run to the top of a tree. In captivity, sentry box cages or pens with nest boxes situated at the top of the cage will allow them to carry out this behaviour (**Figure 3.3**).

Figure 3.3. Top of a marmoset sentry cage showing a nest box

Source: Newcastle University

The need for an element of unpredictability

In the wild, an animal is faced with a changing environment: changes in climate, changes in food and food sources, other species invading their territory, etc. Not all of these changes are desirable, but they do provide stimulation for the animals.

In laboratory animal facilities, the 'climate' has to be constant, but there are ways in which small changes can be introduced to stimulate the animal, provided that the experiments are not compromised. The type of bedding can be changed, alternative balanced food sources can be given,

and items provided to stimulate gnawing and chewing (**Figure 3.4**) can be changed periodically to maintain interest. Periods of training and time to interact with carers can all help to provide this element of unpredictability.

Figure 3.4. Beagle with chew toy

Source: Martin Heath

Opportunity to achieve goals

In the wild, animals are constantly faced with problems that they have to overcome to survive; in captivity, their survival is more certain. However, there are opportunities for them to achieve goals. For instance, products can be given to smaller animals that they can rip up to make nests; this provides them with the satisfaction of achieving a goal and keeps them occupied (**Figure 6.17**). Searching for food in the bedding material or forage boxes mimics the need to hunt for food in the wild, while more complex food puzzles provide non-human primates with the same sort of problems that wild animals face in obtaining food (**Figure 3.5**).

Training programmes can be used in this category too, as they give animals the opportunity to achieve a goal. Dogs can be trained to perform many tasks in the facility, for example walking on a lead (**Figure 3.6**) and walking up a ramp onto a table. Training is good for both the dog and the carer.

Rat playpens have been introduced in some units to give rats the opportunity to climb, balance and explore new environments (Tough, 2023).

Figure 3.5. Food puzzle

Source: Datesand Ltd

Figure 3.6. Dog trained to walk on a lead

Source: Pfizer UK

The need for companionship

Group housing fulfils many of the needs mentioned above. Security is provided by the number of group members, particularly in social species, such as mice and rats, and pack animals, such as dogs. Unpredictability is provided by the changing social relations with cage mates, and births and deaths in breeding colonies. Achievement of goals is provided by grooming, playing, play fighting and rearing offspring. There can be problems, however. Some strains are aggressive, particularly males, and some species are solitary, so an understanding of the strains, sexes and species is essential when group-housing animals (see **Chapter 5.6.1**).

Effects of environmental enrichment

An important point with all environmental enrichment is that it must be compatible with, and not frustrate, the studies that the animals are part of.

It is essential that the effect of an item of environmental enrichment is observed and evaluated to make sure it is fulfilling its purpose. Just looking good is not enough; a rat may be seen throwing an item around the cage and the assumption made that they are enjoying it, when what is really happening is that the rat finds the item annoying and is trying to throw it out of the cage. To carry out a proper evaluation, see the resource from the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), 'Evaluating environmental enrichment'.

3.5. Culture of care

The level of animal welfare that is demanded of people working in laboratory animal facilities can be provided only if a culture of care exists within the organisation.

A culture of care requires that everybody, from the most senior scientist to the most junior staff member, including administrators, whether or not they have direct contact with animals, accepts that all animals must be treated with care, respect and concern and that everything involving animals is approached with those values to the forefront of the mind. A culture of care should be the overriding principle of the establishment.

The law and the Three Rs form the basis of the culture, but they are only the starting points. The law sets the standards required, but they are the minimum acceptable standards and these alone do not indicate a good culture of care. As we have read, Russell and Burch expected people to continually improve and extend the Three Rs. This implies that staff should keep up to date with the latest research and techniques in animal welfare, and they should be encouraged to use this knowledge and their experience in their workplace to develop new ways to improve the lives of laboratory animals while supporting scientific research.

It is the job of the person in the organisation with overall responsibility for ensuring compliance with the Directive to promote the Three Rs and to promote the culture of care. That person is supported by the AWB and the other staff members with specific responsibilities under the Directive (see **Chapter 1**). It is the role of these people to disseminate information, ensure efficient communication, hold training sessions and publicise what the culture of care means so that all members of staff know that they, as individuals, are essential in making the culture work and know how they can positively contribute to it. The junior staff who spend the most time with the animals and deliver the care directly to the animals play a pivotal role by, for example:

— carrying out their duties diligently;

— being constantly observant of each animal's condition so that they pick up problems quickly;

— reporting problems as rapidly as possible so they can be resolved immediately.

Encouraging all staff to suggest advances in the Three Rs and recognising and publicising their contributions encourages others to do the same.

A culture of care goes further than the high standard of animal care that is demanded, because it also demands a high standard of care for staff. Colleagues at all levels must be treated well and should be supported when needed. Laboratory animal work can be emotionally challenging at times and there should be a mechanism in the culture to recognise problems and help the individual to overcome any personal issues.

3.6. Sources of information

It is important for animal care staff to keep up to date with information about the animals they care for. Knowing where to find the information is an important skill. The first place to look is in the workplace. Within each establishment, Directive 2010/63/EU, requires that a person is appointed with the specific responsibility to ensure those caring for animals have access to information about them. A person with responsibility for ensuring education, competence and continued professional development must also be appointed. These people are good sources of information. In addition, internal trainers and experienced colleagues are also good sources of information.

Several journals cover laboratory animal science and technology, and they will be mentioned elsewhere in this book, but one is very relevant to the subjects dealt with in this chapter: the journal of the Institute of Animal Technology (IAT), called Animal Technology and Welfare. It is available, free of charge, online (www.atwjournal.com). It is the official journal not only of the IAT, but also of the European Federation of Animal Technologists. It is written by animal care staff for animal care staff, and often has articles and short communications about environmental enrichment and other related topics. It is written in English, but the abstracts of the major articles are translated into several European languages.

References

Animal Technology and Welfare, https://www.atwjournal.com/.

BioTechniques (2023), 'What is organ-on-a-chip technology?', YouTube website, https://www.youtube.com/watch?v=8tlHd5pYHOY.

Blackburn, S. (2016), Oxford Dictionary of Philosophy, 3rd edition, Oxford University Press, Oxford.

Brambell, R. (1965), 'Report of the technical committee to enquire into the welfare of animals kept under intensive livestock husbandry systems', HM Stationery Office, London, pp. 1–84.

Clemence M & Leaman J (2016). Public attitudes to animal research in the UL 2016. Ipsos Mori. https://www.ipsos.com/sites/default/files/publication/1970-01/sri-public-attitudes-to-animal-research-2016.pdf.

Damon, E. G., Eidson, A. F., Hobbs, C. H. and Hahn, F. F. (1986), 'Effect of acclimatisation on nephrotoxic response of rats to uranium', Laboratory Animal Science, Vol. 36, No 1, pp. 24–27.

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, OJ L 276, 20.10.2010, p. 33, ELI: https://eur-lex.europa.eu/eli/dir/2010/63/oj/eng.

Dolan, K. (1999), Ethics, Animals and Science, Blackwell Science, Oxford.

Dolan, K. (2007), 'The ethical implications of the use of animals in scientific procedures', in: Barnett, S. W. (ed.), Manual of Animal Technology, pp. 326-347 Blackwell Publishing, Oxford.

European Commission (2010), 'Science and technology report – Special Eurobarometer 340', SP340, https://europa.eu/eurobarometer/surveys/detail/806.

European Commission (2023), Commission staff working document – Summary report on the statistics on the use of animals for scientific purposes in the Member States of the European Union and Norway in 2020, SWD(2023) 84 final, Brussels.

Festing, M. F. W. (1994), 'Reduction of animal use: Experimental design and quality of experiments', *Laboratory Animals*, Vol. 28, pp. 212–221.

Harrison, R. (1964), Animal Machines – The new factory farming industry, Vincent Stuart Publishers.

NC3Rs, 'Evaluating environmental enrichment', NC3Rs website, https://nc3rs.org.uk/3rs-resource-library/evaluating-environmental-enrichment.

Russell, W. M. S. and Burch, R. L. (1959; reprinted 1992), The Principles of Humane Experimental Technique, Universities Federation for Animal Welfare (UFAW), Wheathampstead.

Tough, B. (2023), 'Rat playpens – The effect on welfare, technical time and budget', Animal Technology and Welfare, Vol. 22, No 2, pp. 156–157.

Further reading

European Commission, 'Animals in science – The "Three Rs"', European Commission website, https://environment.ec.europa.eu/topics/chemicals/animals-science_en#the-three-rs.

Joint Research Centre, 'EU Reference Laboratory for alternatives to animal testing (EURL ECVAM)', European Commission website, https://joint-research-centre.ec.europa.eu/projects-and-activities/reference-and-measurement/european-union-reference-laboratories/eu-reference-laboratory-alternatives-animal-testing-eurl-ecvam_en.

Robinson, S. and Kerton, A. (2021), 'What does a culture of care look like? Lessons learnt from a workshop survey', LabAnimal, Vol. 50, No 10, pp. 269–271.

Royal Society for the Prevention of Cruelty to Animals (RSPCA) (2020), 'Resources for AWERB members – Promoting a culture of care', RSPCA website, www.rspca.org.uk/webContent/staticImages/Downloads/PromotingACultureOfCare.pdf.

Contribution of this chapter to the Three Rs

The only justification for using animals in scientific research is if it is done in accordance with the principles of humane experimental technique, which were proposed by Russell and Burch in 1959. This chapter outlines these principles and seeks to explain the role of the readers of this book in promoting the care and welfare of the animals. The importance of a culture of care in an animal facility is also covered.

Self-assessment questions

1. Explain what is meant by each of the Three Rs and give an example of each one (do not use an example mentioned in the chapter).
2. What is the difference between relative replacement and absolute replacement?
3. What is meant by control of experimental variables?
4. What is the aim of environmental enrichment?
5. Why is it important to include staff in the culture of care?

4. Maintaining health status

After reading this chapter, you should:

- be able to list the potential disease risks in the animal facility, the predisposing factors that may be relevant and how barriers, containment levels and the use of sentinels can be used to maintain health status (learning outcome 4.9);
- be able to describe how the establishment is organised to maintain the appropriate health status for the animals and scientific procedures (learning outcome 4.5);
- recognise normal or desirable appearance and behaviour of individuals, and signs of positive well-being in the context of species, environment and physiological status (learning outcome 5.1);
- be able to recognise abnormal behaviour and signs of discomfort, pain, suffering or distress, and the action that should be taken if animals are found to be exhibiting those signs (learning outcome 5.2).

4.1. Introduction

Disease in a laboratory animal facility results in suffering of individual animals. If the disease is infectious, the whole colony could be affected, as infections spread quickly where a large number of animals are housed in close proximity.

Many small laboratory animal species are particularly susceptible to infectious disease, as most have been bred and housed in facilities designed to exclude infectious agents, and therefore have had no opportunity to build up natural immunity to any infectious disease.

In addition to causing animal suffering, disease interferes with experimental results because physiological changes in the body caused by the disease alter the response to experimental procedures. The effects of the disease plus those of the experimental procedure will cause an even greater assault on the welfare of the animal and could even result in death.

If disease-causing organisms gain entry to a breeding colony, the results can be devastating. Sick animals may not breed at all, or, if they do, the life of the female will be put at risk. Any young that are born will be weak and may not survive; those that do will not be of use for scientific study. It is essential, therefore, to minimise the risk of disease affecting laboratory animal facilities in order to maintain the high standard of care and welfare expected.

This chapter discusses the causes of disease and how infectious agents spread. It explains how barriers are put in place to inhibit infectious agents getting into laboratory animal establishments and the importance of regular health monitoring of animals to ensure that they are free from disease. It also covers welfare issues and pain.

In addition to this chapter, several other chapters are relevant to maintaining health, specifically, **Chapter 5 'Housing and environment'** and **Chapter 6 'Routines, husbandry and hygiene'**.

4.2. Causes of disease

For the purposes of this chapter, disease can be defined as any condition that interferes with the normal well-being of an animal. Diseases are divided into two main groups: infectious and non-infectious.

4.2.1. Infectious disease

Infectious diseases are caused by specific agents, for example viruses, bacteria, fungi and invertebrate parasites. They can be passed from one animal to another directly (e.g. contact) or indirectly (e.g. in the air, on food, by vectors). Some infectious diseases can be passed from animal to humans; these are called zoonoses (see **Chapter 14.5.1**).

A brief mention should be made of the unusual infectious agents called prions. Prions appear to be abnormal proteins that can cause other, normal, proteins to alter their structure, and therefore alter the structure and function of the tissue they are part of, specifically the brain. The disturbance of the structure results in holes appearing in the brain, giving it a sponge-like appearance when viewed with a microscope, leading to the name given to the group of diseases caused by these agents: transmissible spongiform encephalopathies (TSEs). The individual diseases are probably better known: bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep and Creutzfeldt–Jakob disease (CJD) in humans. The only reason people working in laboratory animal facilities will come across prions is if TSEs are being studied; if this is the case, specific training and safety measures will be in place.

Transmission of infectious disease

Disease-causing agents spread from infected animals to non-infected ones by the following means.

Transmission by infected animals. Infectious agents reproduce in the bodies of infected animals and are then released (or shed) to infect other animals.

In most cases, infected animals will be obvious, as they will have clinical signs that can be clearly seen (see **Chapter 4.5**). However, clinical signs take time to develop after the animal is first infected (this is called the incubation period). During this period, the animal will appear well. In some cases, animals that are usually susceptible to the disease may be infected and be able to pass on the infection to other animals, but never show clinical signs themselves; these are called carriers.

A variety of measures help prevent the entry of infected animals. These will be discussed in later sections of this chapter.

Question: Before getting to that section, try to identify measures that will help prevent the entry of infected animals into an animal facility.

Transmission by vectors. Vectors are organisms that transmit infectious agents but are not affected by them. Humans can act as vectors, bringing infectious agents in on clothes etc. Other examples of vectors are fleas, mosquitoes and ticks, all of which puncture the skin to feed on blood, and can introduce infectious agents directly into the circulatory system. Flies and other arthropods can carry eggs of invertebrate parasites such as tapeworms.

A variety of measures help prevent the entry of vectors into the animal facility. These will be discussed in later sections of this chapter.

Question: Before getting to that section, try to identify measures that will help prevent the entry of vectors into an animal facility.

Transmission by fomites. Fomites are inanimate materials capable of absorbing and transmitting disease-causing agents. Common fomites are food, water, bedding and mops.

A variety of measures help prevent the spread of infectious agents by fomites. These will be discussed in later sections of this chapter.

Question: Before getting to that section, try to identify measures that will help prevent the spread of infectious agents by fomites in an animal facility.

Transmission by intermediate hosts. These are animals in which parasites must spend time in order to complete their life cycle. The cat tapeworm (*Taenia taeniaeformis*), for instance, carries out some of its development in the liver of rodents and is transmitted to the cat when it eats the rodent; another example is the sheep liver fluke (*Fasciola hepatica*), which develops in the body of the water snail (*Galba truncatula* formally known as *Lymnaea truncatula*). When developed, the immature fluke leaves the snail and attaches to grass; sheep become infected when they consume the grass.

This mode of infection is more of a risk if animals are kept outdoors, but good land management and, in some instances, vaccinations and anthelmintic treatment reduce the risk. General disease protection methods (see **Chapter 4.3**) will reduce the risk of intermediate hosts entering buildings housing laboratory animals.

Transmission of human disease to animals. The potential of humans acting as vectors of infectious disease to animals has been mentioned above, as has zoonosis, but there are instances when a disease from which a human is suffering can be passed on to animals. Examples are influenza to ferrets and marmosets, and measles and tuberculosis to non-human primates. Any animal care staff feeling unwell, even if it is a minor condition such as a cold, must report it to their managers before they have contact with susceptible species.

A knowledge of how infectious disease spreads is important because it suggests ways in which transmission can be blocked (see **Chapter 4.4**).

4.2.2. Non-infectious disease

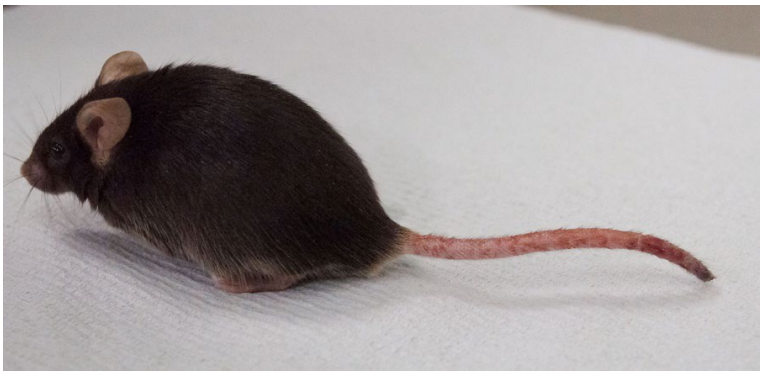
As the name implies, non-infectious diseases (also called non-communicable diseases) cannot be transmitted from one animal to another; they affect only individual animals. However, a number of individuals may be affected at the same time if they are exposed to the same situation. For instance, low humidity and high temperature can cause a condition called ringtail in rodents (see **Chapter 5.6.4**); many animals in the same room may be affected, but they do not catch it from each other. **Table 4.1** gives some examples of non-infectious diseases.

Table 4.1. Examples of non-infectious diseases

Group	Description	Examples
Traumatic	Physical injury	Fight wounds, broken limbs, surgical wounds (**Figure 4.1**)
Nutritional	Insufficient or excess of one or more nutrients	Avitaminosis, obesity, undernourishment
Genetic	— Variety of conditions inherited from parents — Conditions produced as a result of genetic manipulation	Immune deficiency, malocclusion (**Figure 4.2**) Genetic manipulation can result in many detrimental conditions (cancer, diabetes, hypertension, etc.)
Environmental	Reaction to adverse change in environment	Extremes of temperature, noise, low humidity (see **Section 5**)
Metabolic	Failure of normal metabolic processes	Diabetes, hypocalcaemia (decreased levels of calcium in the blood)
Individual infections	Conditions caused by infectious agents, but which are not passed on to other animals	Pyometra (infection of the uterus), infected wounds

Source: Stephen Barnett

Figure 4.1. Traumatic damage to tail



Source: MRC Mary Lyon Centre

Figure 4.2. Overgrown teeth caused by malocclusion

Source: MRC Mary Lyon Centre

4.3. Controlling the passage of infectious agents into and out of animal facilities

In order to protect animals and staff working in laboratory animal establishments the passage of infectious agents must be controlled. This control is achieved by positioning barriers in vulnerable places throughout the facility. In most types of scientific investigation and breeding units the barriers must keep infectious agents away from the animals, these are called bio-exclusion barriers. If the work involves investigation of infectious disease then the barriers must stop the pathogens from escaping and infecting other animals, staff and the wider community, these are called bio-containment barriers.

4.3.1. Bio-exclusion barriers

A bio-exclusion barrier is any physical arrangement, procedure or routine set up with the intent of minimising the likelihood of contamination of the animal with unwanted infectious agents (Clough, 2007).

The number and type of the barriers that are erected in a facility depend on the category of animals being housed there. **Table 4.2** summarises these categories.

Table 4.2. Categories of animals kept in bio-exclusion facilities

Category	Description
Specified (or specific) Pathogen Free (SPF)	Animals that are known to be free of named pathogens. The specific pathogens that animals are free of can be provided by suppliers, who regularly screen their animals. SPF usually refers to small rodents who are kept in Individually Ventilated Cages (IVCs) (see **Chapter 5**)
Germ Free (Axenic)	Germ-free animals are free from all micro-organisms and viruses, including the normal flora in the gut and on the skin. They have to be kept behind very stringent barriers (e.g. in isolators as any infectious agent may kill them)
Gnotobiotic	Gnotobiotic means 'known life'. They start as germ animals and are given one or more microorganisms, depending on what the scientist is studying. These animals need to be housed in isolators or Individually Ventilated Cages (IVCs)
Conventional	These are usually larger species, such as dogs, non-human primates and farm animals. They are kept in hygienic conditions, enclosures are open to the room air (that means they are not in Individually Ventilated Cages (IVCs) or isolators) and staff wear Personal Protective Equipment (PPE) and boots, but there is no showering in. There are barriers to the entry of vermin and unauthorised people. The environment is maintained at the optimal levels for the species.

Source: Stephen Barnett

4.3.2. Embryo transfer rederivation

When a new colony of specified pathogen-free (SPF) or germ-free animals needs to be established from previously conventional animals, or when a disease breaks out within a bio-exclusion colony, new, microbiologically clean, animals are often produced using embryo transfer rederivation.

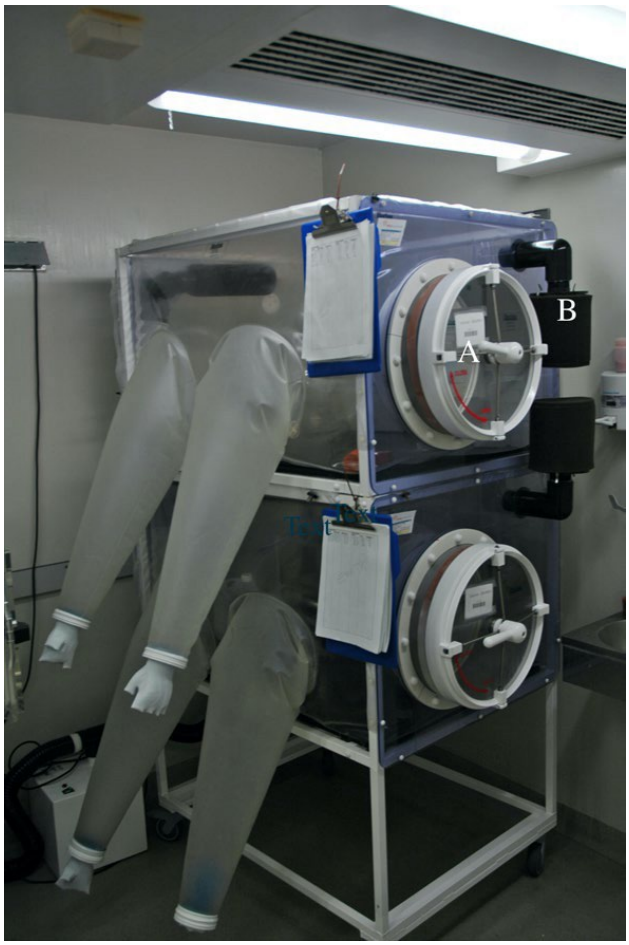
Mating takes place as normal, but at a very early stage of development, the embryos are removed from the donor mother and are implanted into a microbiologically clean recipient or surrogate mother who is housed in a full barrier facility. The recipient mother is pretreated with hormones so the uterus is able to accept the embryos, the recipient mother then carries them to full term, gives birth and rears the young.

4.3.3. Isolators

SPF animals are usually maintained in a unit as described below (**Chapter 4.3.4**). Axenic and gnotobiotic animals can be housed in IVCs, but can also be housed in isolators (**Figure 4.3**). These are constructed of flexible plastic and supplied with air that has been passed through high-efficiency particulate air (HEPA) filters. Isolators are fitted with sleeves with gloves attached at one end; care staff put their arms through the sleeves into the gloves so the animals can be fed, watered, cleaned, etc. On one end of the isolator there is a port, which is a large-diameter, ridged plastic tube with one end inside the isolator and the other end on the outside. Both ends are covered with removable caps.

Before the isolator is used for the first time, it is sterilised with vaporised hydrogen peroxide (VHP) or another suitable agent. Food and other supplies to the isolator and waste from the isolator are passed through the port. Supplies into the isolator are pre-sterilised and double-bagged. The outer cap on the port is removed, the outer bag of the supplies is opened and the sterile inner bag is slipped out into the port; the inner bag must not be touched. The goods in the port are then sprayed with a suitable disinfectant and the port resealed. After 12 hours (often overnight), to give the disinfectant time to work, the inner cap of the port can be removed and the supplies taken into the isolator. Waste is placed in the port and it is resealed from the inside. It is then safe to open the outside to remove the waste. At no time should the two ends of the port be opened together, and, after the outer port seal has been removed, the inner one must not be opened until the inside has been disinfected.

Figure 4.3. Flexible film isolators. A = entry port; B = Filter for air extract

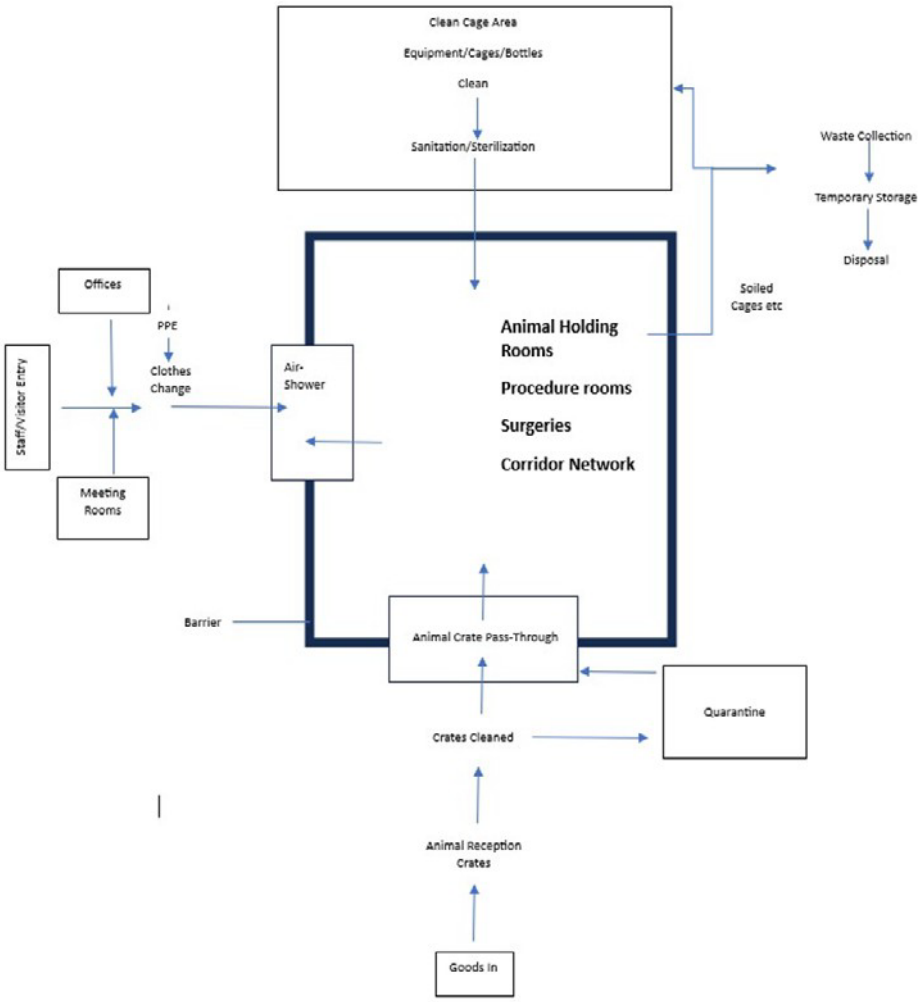


Source: Stephen Barnett

4.3.4. The specified pathogen-free facility

Figure 4.4 is a block diagrammatic representation of a typical SPF laboratory animal facility. At its centre is the area that contains the animal rooms, procedure rooms, store rooms, corridors, etc. There is a barrier around this area which aims to prevent infectious agents from gaining access to and spreading within the area. Most of the barrier is a solid wall, but there must be gaps in the wall to allow air, people, animals and goods in and out. Installations such as airlocks / air showers, autoclaves, VHP chambers and dunk tanks at the entry and exit points, and a filtered air supply, are examples of these types of barrier.

Figure 4.4. Block diagram of a laboratory animal facility



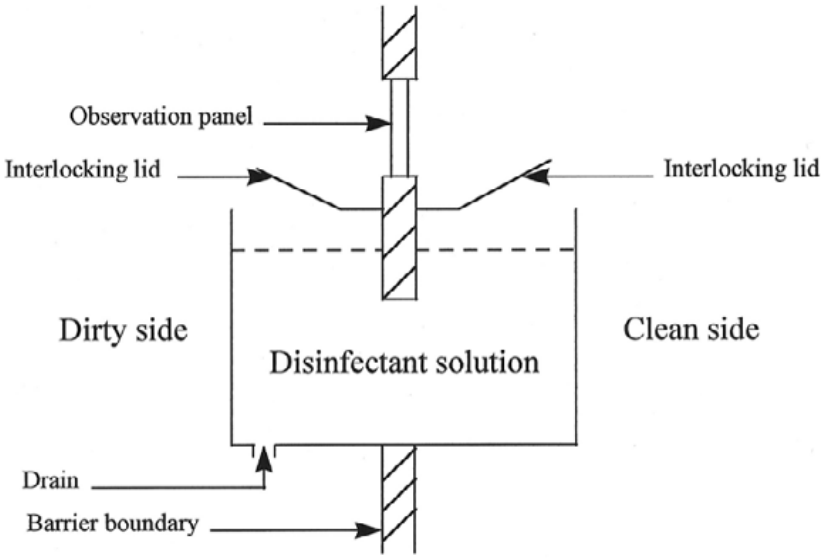
Source: Cube Cleantech Ltd

Airlocks. An airlock is a structure like a cupboard, with two doors that straddle the barrier. The doors are tightly sealed so that no air can leak around the edges. One door opens on the inside of the barrier (the clean side) and the other on the outside (the dirty side). The two doors cannot be opened together or the barrier will be breached. The inner door must not be opened after the outer one has been opened until the area between the two has been disinfected. Airlocks can be of different types. For instance, in some, VHP is used to sanitise material entering the unit (VHP chamber); in others, the airlock is sprayed with disinfectant using a fogger (see **Chapter 6.4.1**).

Autoclave. An autoclave is a pressure vessel that produces steam at greater than atmospheric pressure (and therefore at a higher temperature) to sterilise inanimate materials (see **Chapter 6.4.3**). These can be used to pass supplies (that are not damaged by heat) across the barrier.

Dunk tanks. Like airlocks and autoclaves, these are situated through the barrier. They are large tanks that contain disinfectant. The tank is partly divided by the barrier wall, which descends lower than the level of the disinfectant (**Figure 4.5**). Pre-sterilised materials (e.g. previously irradiated pelleted diet) that have been double-bagged can be passed through by slitting the outer bag and, without touching the inner bag, slipping it under the disinfectant. After a period of time to ensure the disinfectant has destroyed any pathogens on the outside of the bag, it can be retrieved by staff within the barrier.

Figure 4.5. Disinfectant dunk tank straddling the barrier between the clean and dirty sides



Source: Robert W Kemp

4.3.5. Entering the facility

All outer doors to the facility are protected with barriers to stop rodents and flying insects (**Figure 4.6** and **Figure 4.7**) entering. Contamination control floor mats are placed at the entrance to clear contamination from the soles of the foot ware of people entering.

Figure 4.6. Insect barrier

Source: Stephen Barnett

Figure 4.7. Rodent barrier

Source: Stephen Barnett

Only approved people enter through an outer door, and, unless they work in the unit, they do not progress beyond the office. The remaining barriers operate as follows.

Staff entry and exit. Staff change out of their outdoor clothes, cover their underclothes with sterile coveralls and enter an air shower for the prescribed time (**Figure 4.8**). Alternatively, they wet-shower and then, when dry, dress in sterile scrubs, boots, hairnets, masks and gloves. On leaving the animal block, staff exit through the air shower to remove animal dander from their PPE, or via a separate part of the same barrier, where there is no need to shower.

Animal entry. Animals destined for an SPF facility must come from approved SPF breeders, must have a prescribed negative microbiological screening report and must have been transported in SPF-grade transport containers. The animal containers are disinfected and pass through an airlock into the unit. Animals whose health status is not known are first put into the quarantine unit. The quarantine unit is isolated from the main SPF unit so that animals in the SPF unit are protected from infection. Animals can be kept in quarantine for a time (to see if they develop disease) or until they have a series of negative health screen results indicating that they are of suitable health status to join the main SPF unit. Alternatively, the animals are rederived; this means that they are mated in the quarantine unit and then the process described in **Chapter 4.3.2** is carried out.

Other supplies. Food is usually pre-sterilised by irradiation (see **Chapter 6.4.3**). It is double-bagged so that when it arrives at the facility the outer bag can be slit open and the inner bag can be slipped out into a dunk tank, VHP chamber or disinfection chamber. It is important to ensure that the inner

bag is not touched during this process. Food can be autoclaved into an SPF facility, but it must be specially formulated, as some nutrients will be depleted by the heat produced in the autoclave. Other supplies can be autoclaved in or passed through a VHP chamber or disinfectant chamber.

Dirty cages. Soiled cages are transferred to the dirty side of the cage wash section (see **Chapter 6.2.1**). Racks of cages are covered with trolley covers to prevent the escape of pathogens and allergens on the journey (**Figure 4.9**). The soiled bedding is removed in a cage dump station and sent for disposal; the cage is then passed through the cage washer (then autoclaved if necessary) back into the unit.

Figure 4.8. Air shower

Source: Tecniplast UK

Figure 4.9. Trolley covers

Source: Tecniplast UK

Airflow. The air distribution in an animal facility is arranged so that the air blows any airborne infectious agents away from the animals. However, another factor has to be considered: laboratory animal allergens (LAAs) (see **Chapter 14.4.2**). These substances produced by the animals can cause serious allergic reactions in humans, so they must be controlled in the facility and prevented from escaping. When rodents are housed in IVCs, the primary barrier is the cage. The system protects the animals from airborne infectious agents and prevents allergens escaping from the cage to the room.

The rooms of the facility are kept at positive pressure. The relative pressures are as follows.

— The entrance corridor system is at higher pressure than outside (ambient pressure), so pathogens from the outside cannot enter.

— If animals are housed in IVCs, the animal holding rooms and procedure rooms are at a lower pressure than the IVCs and the access corridors. This prevents any pathogens and allergens from being released through the unit. Air that is extracted from the IVCs is passed through HEPA filters, which remove all the contaminants picked up from the cage before releasing the air into the room.

— The cage-cleaning area is at deeply negative pressure, compared with the corridor system, to ensure that no animal and waste-borne microorganisms from the soiled caging escape back into the corridors and animal rooms. Human changing rooms are at negative pressure for the same reasons.

For more information on bio-exclusion barriers, see Kemp (2007).

4.3.6. Bio-containment

Bio-exclusion facilities do all they can to keep pathogens from getting to the animals; bio-containment facilities do all they can to prevent pathogens from getting out and infecting nearby animals and people. There are four bio-containment hazard groups, listed in **Table 4.3**.

Table 4.3. Biological agent hazard groups

Hazard Group	Description
1	A biological agent that is unlikely to cause human disease
2	A biological agent that can cause human disease and might be a hazard to workers. It is unlikely to spread to the community. An effective prophylaxis or treatment is usually available
3	A biological agent that can cause severe human disease and presents a serious hazard to workers. It may present a risk of spreading to the community, but an effective prophylaxis or treatment is usually available
4	A biological agent that causes severe human disease and is a serious hazard to workers. It may present a high risk of spreading to the community. There is usually no effective prophylaxis or treatment

Source: Advisory Committee on Dangerous Pathogens (ACDP) HMSO 1995

Bio-containment units house animals that may carry pathogens that would be a risk to other animals in the facility. The quarantine unit, described above, is an example of this type of unit. Bio-containment units also house animals that are being used to study biological agents that are a risk not only to other animals but also to the people working with them. If they are not contained, these agents will be a risk to other people working in the facility and to the wider community. Working in this type of environment clearly has serious risks, so the EU has established rules to protect the health and safety of workers exposed to biological agents, by which it means microorganisms, cell cultures and human endoparasites. These rules are laid down in Directive 2000/54/EC. Member States have incorporated these into their national legislation; therefore, the exact requirements may vary slightly between member states.

For each category of risk (see **Table 4.3**), there is a corresponding level of containment. It is beyond the remit of this book to deal with all of them in detail, but the main aim is to protect the workers and other people who may be affected by the pathological agents. Rodents will be housed in IVCs and larger species may be housed in isolators; in both cases the environment can be controlled and extracted air is passed through a HEPA filter to remove airborne pathological agents before being released to the outside. All waste material from the cages will be autoclaved out of the facility before being incinerated.

The rooms that hold animals in the hazard groups are kept at negative pressure to prevent the harmful agents from escaping. Entry to the facility will be strictly restricted to essential personnel only, who will be protected by appropriate vaccinations, when possible, and their protective clothing will include gloves, gowns, respiratory protection and/or positive-pressure ventilation suits (depending on the risk of the agent being used) to prevent them coming into contact with the pathological agent. Showering out of the facility is essential. The facility is designed to be fumigated when studies change or accidents occur (for more detail on biocontainment facilities, see Gerson, 2007).

4.4. Animal health screening programmes

Even though bio-exclusion barriers work efficiently, they do break down occasionally. To make sure a breakdown is identified as quickly as possible, health screening programmes and microbiological monitoring are carried out.

Animal health screening programmes can identify infectious agents before any clinical signs show in the animal, and are also able to identify the presence of infectious agents that may affect experimental results but do not cause any observable clinical signs. They can also prevent infected animals from being supplied from a breeder to another establishment and so passing on an infection.

At a set time, which is dependent on the species and the level of the animal's health status (but is often every three to four months in the case of rodents), screening samples are obtained and sent to a laboratory for testing.

While animal health screening programmes can be very expensive, they are essential in helping to ensure the health status of the animals and helping to ensure the validity of experimental results by reducing variables caused by infections, especially when the animal's immune system is a factor in the study.

4.4.1. Collecting samples for health screening

Screening samples can be obtained directly from the animals or from the animals' environment.

Sampling from animals

Animals for testing are taken from the colony and are sent to specialist microbiological laboratories to be tested for the presence of infectious agents. Animals that are housed specifically for health screening purposes are called sentinels; they will be the same strain as, or a similar strain to, the main colony and kept with them, so, if infectious agents are present, the sentinel will pick them up.

Environmental health monitoring

Environmental health monitoring (EHM) involves the collection and pooling of faecal samples and sampling from the air environment, such as air outlets from IVC racks or air extract ducts. These samples are then tested for infectious agents in a way similar to that of testing samples from animal tissue.

EHM can achieve the same result as sampling directly from animals, without the need to kill animals. This is a valuable example of a replacement technique, as proposed by Russell and Burch (see **Chapter 3.4.1**). For further information on EHM, see NC3Rs (2021) and The 3Rs Collaborative (n.d.).

4.5. Inspecting individual animals for their health and welfare status

Annex III, paragraph 3.1(b), of Directive 2010/63/EU (the Directive) makes it mandatory that all animals are inspected at least once a day. If animals are undergoing procedures, detailed inspections may be required more often.

The observations should consider health, welfare and pain. Some changes in behaviour are common to all three of those conditions; other signs are more specific.

Animals indicate that they are unwell, stressed or in pain by changes in their normal behaviour. To detect these changes, it is necessary to have a knowledge of the normal behaviour of the species and, ideally, the individual animal concerned. This is gained by spending time working with animals and observing their normal behaviour when they are in their cages, moving, eating, drinking, interacting with other animals, building nests, etc. Divergence from the norm could indicate a problem and the animal should be inspected more closely.

All staff, no matter how inexperienced, should watch for signs that indicate an animal is unwell and, if any are seen, report their observations to an experienced colleague.

Initially, animals should be observed from a distance so they are not influenced by the presence of the observer.

An animal that is healthy and at ease will be alert, interested in its surroundings, well groomed, moving easily and eating and drinking normally; its nest will be well maintained and it will be interacting with its cage mates (**Figure 4.10** and **Figure 4.11**). There will be signs of normal faeces in the cage and no signs of blood or other discharges.

Inspection of the enclosure can be as revealing as inspecting the animal. Some of the factors that should be noted are shown in **Table 4.4**.

Figure 4.10. Healthy, alert rabbit in its cage

Source: Stephen Barnett

Figure 4.11. Healthy, alert rat in its cage

Source: IAT

Table 4.4. Signs that could indicate ill health

	Factor	Possible abnormality
Animal	Overall body	Loss of weight, lack of reaction to stimuli
	Movement	Abnormal gait, lack of movement
	Posture	Hunched, head or limb held at an odd angle
	Isolation	Separation from other animals in the cage, pen or field
	Coat condition	Hair rough and erect (referred to as a staring coat), lack of grooming
	Respiration	Rate abnormal, breathing laboured or noisy
	Temperament	Signs of apprehension and aggression in an otherwise docile animal
Enclosure	Food	Ignored, mouthed (food taken into the mouth and then dropped so it is wet), excessive intake
	Water	Ignored or excessive intake
	Faeces and urine	Excessive amounts, too little, diarrhoea, presence of blood
	Discharges	Haemorrhage (blood), mucus, vomit (note that rodents, rabbits and horses cannot vomit)
	Hair	Excessive hair in the cage

Source: Stephen Barnett

4.5.1. Detailed health inspection

In addition to the constant observations described above, it is necessary to carry out more thorough inspections of individual animals on a regular basis. All laboratory animal facilities will incorporate health assessments into their routines. The regularity and nature of the inspection will depend on a number of factors, for example the species, if they are undergoing or are about to undergo scientific procedures, or if they are being selected for breeding or for a routine inspection.

Although there may be some variation in the way inspections are carried out, the basic principles are common to all mammals. It is necessary to follow a routine so that no point is overlooked. Any animal giving cause for concern, however trivial, should be reported to a senior member of staff without delay.

As mentioned before, the inspection starts when the animal is in its enclosure (e.g. cage, pen), where it can be observed undisturbed. Movement, posture and respiration can be noted without disturbing the animal. Respiration rates of small rodents may be too fast to record accurately without special equipment, but in larger species it is much easier. **Table 4.5** shows the typical respiration rates of common laboratory species.

Table 4.5. Ignored or excessive intake

Animal	Respiratory rate (breaths per minute)
Mouse	180
Rat	80
Rabbit	55
Dog	25
Cat	26
Rhesus monkey	35

Source: Flecknell (1996)

The enclosure should also be inspected at this point to ensure that none of the signs mentioned in **Table 4.4** are present.

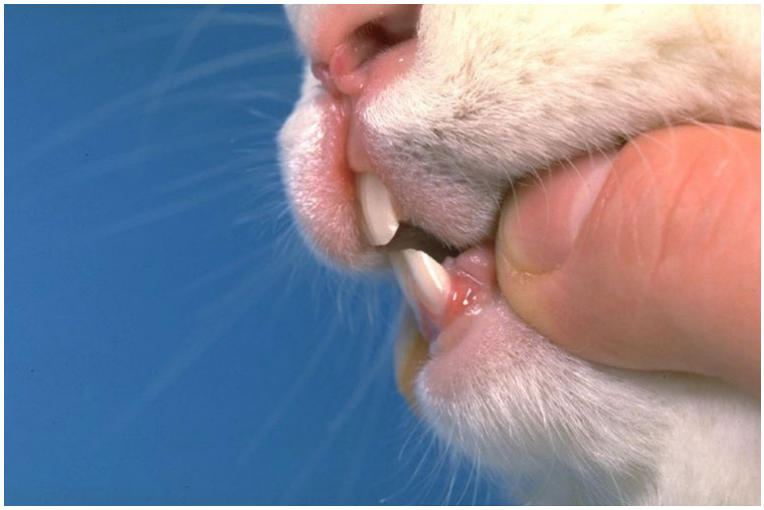
For close inspection (after observing the animal in its cage), individual animals should be removed from the cage by a method appropriate for the species (see **Chapter 13**), and larger species should be placed on a non-slip surface where they will feel safe. The inspection should follow a logical sequence, for instance beginning with the head, working down the upper surface of the body and continuing to the underside.

The following example applies to a rabbit; see **Table 4.6** for a checklist suitable for a mouse.

The mouth

Lips, gums and tongue should have a clean appearance; all teeth should be present and aligned properly. Examples of abnormalities that could be seen are lesions (sores, cuts); inflammation (redness, swelling and heat); and misaligned (malocclusion), overgrown (**Figure 4.12** and **Figure 4.13**), broken or missing teeth. Soreness or wetness on the chin could indicate problems in the mouth.

Figure 4.12. Normal teeth of a rabbit



Source: Newcastle University

Figure 4.13. Bottom incisor overgrown

Source: Anthony Buckwell

The nose

The nostrils and surrounding area should be clean and clear. If the animal has a respiratory infection, there may be signs of discharge causing blocked nostrils and matted fur. Rabbits and rodents wipe their noses with their paws, so matted fur may also be found on the inside surface of their forelegs (**Figure 4.14**).

Figure 4.14. Rabbit with respiratory disease. Note the discharge around the nose and the matted fur on the forepaws

Source: Newcastle University

The eyes and eyelids

The eyelids should be clean. Any signs of swelling, redness or discharge of any kind should give cause for concern. The eyes should be clear and bright with no sign of redness or discolouration in the sclera (the white of the eye) or cloudiness in the cornea.

If a problem is found in one eye, it could be due to a foreign body or traumatic damage. If a problem is found in both eyes, it is more likely to be an infection.

The ears

The shape, thickness and size of ears, and the amount of fur they carry, differ widely between species, so the normal appearance will differ too. In general, ears should be clean and alert (responding to the source of sound). There should be no sign of traumatic damage that could be caused by fighting or scratching. Ear mites colonise all species if they get the chance; they cause intense irritation and the animals scratch in response. Mites can cause hair loss, thickening of the skin and discharges that become encrustations. Signs may be on the edges of the ear or deep down inside, depending on the type of mite.

An example of an ear mite that may infest rabbits is the rabbit ear canker mite (scientific name *Psoroptes cuniculi*). A yellow/brown crust deep inside the ear may indicate the presence of this mite (**Figure 4.15**).

Figure 4.15. Brown/yellow exudate in the inner ear indicating ear canker

Source: Stephen Barnett

The body, coat, back and sides

Hands or fingers are passed over the back and body of the animal to check for lumps and bumps, covered by fur, that could indicate abscesses, cysts or wounds. This process may also indicate weight loss (because bones are easily felt). The coat should be well groomed. Fur consists of two types of hair: rough guard hair and softer downy hair. Brushing the coat the wrong way (from tail to head) in thickly furred species allows the downy fur to be inspected. If it is less dense than it should be, it could indicate the presence of body mites, which tend to affect the softer downy hair.

The loose skin on the back should be gently pinched up and allowed to fall (a process called tenting). In a healthy animal, the skin should return to normal quickly; if it takes a long time, the animal may be dehydrated.

The tail and anus

At this point smaller species may need to be turned over to inspect the underside of the body.

The anus and the region surrounding it should be clean. There should be no faecal staining or evidence of discharge, blood or prolapse (which is when the wall of the rectum protrudes through the anus).

The genitalia

Females. The vulva should be clean with no discharges or traumatic damage. However, the appearance varies during the oestrous cycle, so what is observed must be correlated with the stage of the cycle the female is in. Oestrous cycles are discussed in the chapter on breeding (see **Chapter 8.2**).

Males. Sexually mature males should have two well-developed testes. Occasionally, one or both do not descend into the scrotum. In some species, injury to testes may result from keeping males together. The penis should show no signs of inflammation and the prepuce (foreskin) should be clean.

The hind legs

The hind legs should be checked for swollen, damaged or stiff joints. The feet should be checked for broken, torn or overgrown claws. In some species, pressure sores are occasionally seen, especially when animals are housed on grids (which are not recommended), for example sore hocks in rabbits.

The abdomen, thorax and axillae

The fur is thinner on the underside of the body in many species; therefore, some conditions may be easier to see, for example scratches, hair loss and inflammation. It may also be easier to see evidence of parasites. In breeding females, mammary glands should be checked to ensure that there are no signs of lumps or inflammation that could be due to mastitis or tumours.

The forelegs

These should be checked as described for the hind legs.

Table 4.6. Health checklist for a mouse

1	Observe the mice in the cage before opening the lid, looking for abnormal behaviour, for example sitting on their own, limping (abnormal gait) or any obvious abnormalities
2	Hold the mouse and, starting from the head and working backwards, check the following: — nostrils (nares) dry and clear with no discharge; — whiskers clean and dry; — front teeth (incisors) aligned and not overgrown (malocclusion); — ears (pinnae) clean, no damage; — fur glossy and smooth, not sticking up (staring); — body mass normal, neither thin nor fat; no lumps or bite marks; — anus clean, with no staining with urine or faeces; — genitals normal; — feet clean; — nails clean, not overgrown; — tail straight, no damage

Source: Ken Applebee

4.5.2. Example of a non-mammalian species: zebrafish

Zebrafish are used as an example of a non-mammalian species in this chapter because fish are the second most commonly used type of laboratory animal, and zebrafish make up 65% of all fish used. The same principles of health assessment mentioned for mammals apply to fish: identification of ill health is based on a deviation from normal appearance and behaviour. Healthy fish will be active in the tank, eating and interacting with other fish (in the wild they travel in shoals). Like mammals, they are susceptible to both infectious and non-infectious diseases. Efficient water treatment equipment is essential to eliminate infectious organisms and to ensure water quality remains high. **Table 4.7** lists some signs of ill health in fish, with some possible causes.

Table 4.7. Some signs of ill health in zebrafish

Sign	Possible cause
General signs: pale, loss of colour, lack of movement, sitting on bottom of the tank	-
Damage to and/or reddening of fins	Could be due to fighting (fin nipping) or bacterial infection
Lesions on the body	Could have infectious cause or problems with water quality (e.g. pH levels)
Protruding scales	Could be early sign of dropsy[1] (**Figure 4.16a** and **Figure 4.16b**)
Red, swollen abdomen	Later sign of dropsy[1]
Enlarged abdomen	Could indicate female is egg-bound (may happen when no male is present)
Loss of balance	Usually infection in swim bladder

Source: Nicola Goodwin

Figure 4.16a. Normal zebrafish

Source: Nicola Goodwin

Figure 4.16b. Fish with dropsy, showing enlarged abdomen and raised scales

Source: Nicola Goodwin

Microbiological monitoring is carried out in zebrafish facilities. For instance, tests are carried out for mycobacterium species (which cause fish tuberculosis, a zoonosis) and microsporidiosis, a fungus-like protozoan that is associated with emaciation in fish (known as skinny disease).

[1] Dropsy is the name given to the condition in which there is an accumulation of fluid in tissues and body cavities. It is not a specific disease, but a clinical sign that can have many causes.

4.5.3. Stress

Daily inspections for health status should also include checking for general welfare problems. If welfare is compromised, an animal will show signs of stress. Stress may accompany ill health, but also occurs in many other situations, a few examples of which are listed in **Table 4.8**.

Table 4.8. Examples of stress-inducing factors

Environmental factors	Noise Vibration Transport Boredom High stocking densities Single housing of social species
Experimental and husbandry factors	Cage cleaning Sudden change of diet Regulated procedures Poor handling and restraint Bullying by cage mates Change of carer

Source: Stephen Barnett

Stress occurs when an animal is exposed to worrying or potentially dangerous situations. It is a normal response and occurs in all animals, including humans. Once the perceived concern has passed, the stress should disappear. Problems arise when the stressful conditions persist or are extreme; then stress can develop into distress. The animal's behaviour and physiology are affected in ways that are similar to those described in health monitoring (see **Table 4.4**).

Signs include the following:

— Reduced resistance to disease.

— Overeating or refusing to eat.

— Lack of grooming or over-grooming.

— Chromodacryorrhoea, a red discharge seen around the eyes and nose of rats. It is released as a result of stress and becomes more noticeable with the severity of the stress.

— Exhibiting stereotypies (repetitive behaviour patterns with no obvious purpose):

- pacing,
- bar chewing,
- over-grooming, including barbering (when a dominant animal gnaws the hair from subordinates), tumbling.

4.5.4. Pain

It is difficult to accurately assess the degree of pain that an animal suffers because, unlike humans, they cannot verbalise how they are feeling. Initially, it is necessary to rely on behavioural signs to indicate that something is wrong. Some abnormal behavioural signs are the same as those of ill health and stress, such as failure to eat and drink, failure to groom, lack of movement, isolation from cage mates and aggression, particularly when picked up. Others are more specific: moving with a limp, sitting with an arched back, licking the painful area or, in extremis, gnawing at the affected area. The degree of pain an animal is suffering is indicated by its facial expressions. Grimace scales, that help interpretate the expressions, have been prepared for mice, rats and rabbits (see NC3Rs, 2022).

Routine daily welfare inspections are designed to identify any animal whose welfare is compromised. To be sure that everything is covered and everybody follows the same method, checklists are often used. The results of these daily inspections should be written down so that they form a welfare record of the animals over time.

More detailed monitoring and accurate records are essential when animals undergoing procedures are being monitored. All the possible adverse effects must be recorded and the degree of suffering and distress must be assessed. In this case, scoresheets are used, prepared by the senior scientist, to help quantify the harmful effects the animal is feeling.

4.6. Action to be taken after completing the welfare inspection

The whole point of daily welfare inspections is to identify any animal whose welfare is compromised so that the problems can be rectified. It is essential that problems are reported in accordance with local rules as quickly as possible. Some conditions, such as overgrown claws in rabbits, may be able to be ameliorated (improved or made better) by relatively minor interventions, but it should be emphasised that any procedures should be carried out only by trained and authorised staff.

If the animals being monitored are part of a project, any concerns have to reported to the person responsible for the project as quickly as possible.

Analgesics (drugs that relieve pain) and related medicines may be appropriate, but the veterinary surgeon or the person in charge of the project is responsible for their administration.

References

Clough, G. (2007), 'The environment', in: Barnett, S. W. (ed.), Manual of Animal Technology, Blackwell Publishing, Oxford.

Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC), OJ L 262, 17.10.2000, p. 21, ELI: http://data.europa.eu/eli/dir/2000/54/oj.

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, OJ L 276, 20.10.2010, p. 33, ELI: https://eur-lex.europa.eu/eli/dir/2010/63/oj/eng.

Flecknell, P. (1996), *Laboratory Animal Anaesthesia – A practical introduction for research workers and technicians*, 2nd edition, Academic Press, San Diego, CA.

Gerson, P. (2007), 'The isolation of laboratory animals that are a potential human risk', in: Barnett, S. W. (ed.), *Manual of Animal Technology*, Blackwell Publishing, Oxford.

Kemp, R. W. (2007), 'Animals with a defined microbiological status', in: Barnett, S. W. (ed.), *Manual of Animal Technology*, Blackwell Publishing, Oxford.

NC3Rs (2021), 'Environmental monitoring for rodent health surveillance', NC3Rs website, https://nc3rs.org.uk/events/environmental-monitoring-rodent-health-surveillance.

NC3Rs (2022), 'Welfare assessment – Grimace scales', NC3Rs website, https://nc3rs.org.uk/3rs-resources/grimace-scales.

The 3Rs Collaborative (n.d.), 'Rodent health monitoring', The 3Rs Collaborative website, https://3rc.org/health-monitoring/.

Further reading

Baumans, V. (2005), 'Science-based assessment of animal welfare: Laboratory animals, *Revue scientifique et technique*, Vol. 24, No 2, pp. 503–513.

Latham, N. (2010), 'A brief introduction to welfare assessment: A "toolbox" of techniques', in: Hubrecht, R. and Kirkwood, J. (eds), *The UFAW handbook on the care and management of laboratory and other research animals*, 8th edition, Wiley-Blackwell, Chichester, pp. 76–91.

Contribution of this chapter to the Three Rs

This chapter contributes to both the reduction in the number of animals used and refinement.

Reduction. Establishing barriers to prevent the entry of pathogens greatly reduces the number of animals that may die as a direct result of infectious disease or have to be killed to prevent the disease spreading to nearby colonies.

Refinement. Establishing barriers also protects animals from suffering from infectious disease. Recognising the signs of both infectious and non-infectious diseases, stress and pain enables these to be identified rapidly so that they can be reported and remedial action can be taken.

Self-assessment questions

1. What is the purpose of establishing ‘barriers’ in laboratory animal facilities?
2. Describe the procedure for a member of staff to enter an SPF facility.
3. Describe the procedure for carrying out a daily welfare check on a laboratory animal.
4. List six indications that an animal’s welfare is compromised.
5. What action would you take if you found an animal whose condition gave cause for concern?

5. Housing and environment

After reading this chapter, you should be able to describe suitable environmental and housing conditions for laboratory animals, be able to describe how conditions are monitored and be aware of the consequences for the animals of inappropriate environmental conditions (learning outcome 4.2).

You should also appreciate that changes to or disruption of circadian rhythms or photoperiods will affect animals (learning outcome 4.3).

5.1. Introduction

This chapter covers two related subjects. The first is housing, that is, animal enclosures (cages and pens), and the rooms they are placed in. The second is the environment, which covers all the living and non-living factors that surround the animals and exert an effect on them. If the housing and environment are not maintained at the optimum levels, they can cause serious welfare issues and will adversely affect the validity of experimental results.

5.2. Animal enclosures and housing

Laboratory animals spend almost all their lives in enclosures, so it is essential that their accommodation is designed to provide for their physical needs and as many of their natural behavioural needs as possible. This section considers enclosure design, bedding and nesting materials.

5.2.1. Enclosure design space and stocking density

An enclosure has to provide enough space for the occupants together with bedding and nesting material and appropriate environmental enrichment. Annex III of Directive 2010/63/EU (the Directive) states the minimum floor areas and cage heights for species at various stages of life. The sizes stated in the annex are mandatory minimums; larger cage sizes with greater floor area per animal are permitted and encouraged.

It is the responsibility of animal care staff to ensure that cages do not become overstocked. Overcrowding can happen easily; for instance, a cage containing a correct number of young animals to begin with may soon become overstocked simply because the animals have grown. However, with good husbandry practice this should never happen. Overcrowding is one of the causes of stress in animals (see **Chapter 4.5.3**).

Adequate space is not enough on its own; thought must be given to what goes into the space to enable the animals to use it all. Cage design must reflect the natural behaviour of the animals. For example, rats have a need to rear on their hind legs (bipedal behaviour) to view their surroundings, so the cage height must accommodate this. Rabbits and dogs need to rest above the ground, so a shelf or other structure must be provided (**Figure 5.1** and **Figure 5.2**). Marmosets nest in trees and climb high if they feel threatened, so their cages or pens need to have nest boxes set at the top of a high cage with the means to climb fitted (**Figure 3.3**).

Figure 5.1. Rabbit on a raised platform

Source: Sarah Lane

Figure 5.2. Dog sitting on a raised shelf

Source: Pfizer UK

The enclosure must also provide the occupants with easy access to food and water. In addition to the requirements listed above, cages should:

— provide for the safe containment of the animals;

— be easy to use and service, for example be easy to clean and to provide animals with food and water;

— allow the safe removal and return of the occupants;

— enable animals to be seen so that their welfare can be assessed;

— be able to withstand the attention of their occupants and the routine wear and tear (e.g. cage wash, autoclave, disinfectants) to which they will be exposed;

— meet experimental requirements.

When there is difficulty in providing for all behavioural or experimental needs in one enclosure, other measures have to be taken. For example:

— it is both impractical and inadvisable to provide enough space in a dog's pen for both its housing and exercise requirements, so it is necessary to provide an exercise area nearby and to build an exercise period into the facility routines (**Figure 5.3**);

— if a 24-hour urine sample is required, the animal may need to be housed in a metabolism cage, which is generally of smaller dimensions than the required minimum enclosure size (see **Chapter 11.4.5**).

Figure 5.3. Dogs being exercised in a facility corridor

Source: Martin Heath

5.3. Enclosure material

A variety of materials are used to manufacture cages and pens for laboratory animals. Consideration of the physical properties of these materials helps to assess their usefulness for particular purposes. It is also necessary to decide which cleaning methods are most appropriate to use on them. Physical factors that should be considered include the following.

— Strength: rigidity and ability to resist animal activity and normal wear and tear in the animal unit. For example, metals are very strong, so stainless steel is able to contain Old World primates. Plastics are less strong but they weigh less and make less noise when moved, and they are suitable for housing smaller species.

— Density is defined as mass per unit volume. It is a useful measure to compare the heaviness of objects of different shapes. Metals are much denser than plastics (e.g. the density of stainless steel is approximately 8 g/cm^3, whereas that of polysulfone is 1.25 g/cm^3).

— Thermal conductivity: the ability of a material to conduct heat. Metals are good conductors and therefore conduct heat rapidly. Plastics and wood are poor conductors; poor conductors are good insulators. Where environmental temperatures vary widely, thermal conductivity is of great importance. In modern temperature-controlled laboratory animal facilities, the thermal conductivity of a material used in a cage is a less important welfare consideration; however, plastic does have the advantage of providing a quieter and less industrial feel to the environment.

— Reactions to acids, alkalis and detergents at concentrations encountered in animal units (e.g. descalers, detergents and disinfectants). The materials used to construct cages and pens must be able to resist any damaging effects of these agents, unless they are considered disposable.

— Ability to withstand repeated autoclaving.

Most cages and pens are constructed from metal or plastic or a combination of both. Properties of commonly used caging materials are summarised in **Table 5.1**.

Table 5.1. Properties of common caging materials

Material	Properties	Examples of uses
Wood and wood products (including cardboard)	Construction of cages and boxes is easy with wood; however, wood is difficult to clean and impossible to sterilise, and may rot. Wood is a good insulator, but animals may gnaw it.	Limited use in the animal unit other than in non-human primate caging. It can be used for travelling boxes, shelves and perches
Metals in general	Metals are strong and easily cleaned, and will withstand autoclaving. They are good conductors of heat. They are heavy in comparison with plastics. They usually require specialist manufacturing techniques to construct. They generate noise when dropped or rattled. Galvanised mild steel and aluminium were common in animal facilities, but are less popular now, except for farm animals, for which galvanised mild steel is still used. Stainless steel is more widely used.	
Stainless steel	Very strong material that is resistant to most substances found in animal units. It is very	Large cages for non-human primates (**Figure 5.4**). Racking, cage doors and tops, food

	long-lasting and the most expensive material used for caging. Several grades of stainless steel are available for different purposes (e.g. grade 316L is used for bottle spouts and housing for aquatics; the lower-grade 304L is used for racking)	hoppers and water bottle spouts. It is also used for IVC plenums
Plastics in general	Lightweight materials that are good insulators with variable resistance to damage by chemicals. Plastics make much less noise than metal, both in use and when dropped. Plastic cage bases are made in one piece and are easy to stack. Traditional plastics become brittle with repeated autoclaving and exposure to chemicals, but modern plastics that are tolerant of high temperatures and/or chemicals are much more resistant	
Polycarbonate	A clear plastic that will withstand autoclaving at 121°C. It becomes discoloured and brittle with repeated treatments. Detergents and water denature the material over time	Used for conventional rodent boxes (**Figure 5.5**)
High heat polycarbonate	This material withstands autoclaving to 132°C; otherwise, it is similar to polycarbonate	
Polyethylene (polyethylene terephthalate (PET))	This material will melt at 93°C, so cannot be autoclaved. Structures made from it are	Disposable rodent cages and cage liners
Polysulfone[2]	Transparent material. Withstands autoclaving at temperatures of up to 140°C. Heat and chemical resistant	Cage bases
Polyphenylsulfone*	Very high temperature and chemical resistance	Cage bases
Polystyrene	Cheap and disposable. It is brittle and cannot be autoclaved	Disposable cages
Glass	Transparent and impervious to all chemicals used in the animal facility. It is fragile and is easily broken, resulting in dangerous sharp edges	Metabolism cages

Source: Stephen Barnett after PJKelly, KGMillican and Pam Organ

[2] Polysulfone and polyphenylsulfone are more expensive than polycarbonate cages, but their heat and chemical resistance mean that they have a longer life than other plastic materials. Polysulfone has a life of over three years and polyphenylsulfone of eight years.

Figure 5.4. Large non-human primate cage

Source: Wendy Steel

Figure 5.5. Polycarbonate mouse cage

Source: Stephen Barnett

5.4. Development of enclosure design

The design of enclosures has evolved over time to the benefit of the animals housed in them and the people who work with them. The most significant design development in the last 30 years has been the introduction of Individual Ventilated Cages (IVCs), which now house most of the small rodents used in research. IVCs are sealed cages fitted into racks that carry the conditioned air supply directly to, and extract air directly from, the cage (**Figure 5.6** and **Figure 5.7**). The air can be supplied at positive or negative pressure (see **Chapter 5.6.2** for an explanation of these terms). If the animals are microbiologically clean and need to be protected from external organisms, the IVC is kept at positive pressure. If the animals are being used to study harmful organisms, the IVCs can be maintained at negative pressure. IVC units also help to minimise the dispersion of Laboratory Animal Allergies (LAAs) into the environment (see **Chapter 14.4.2**). Systems are available to monitor food and water consumption, dampness of bedding, animal activity, etc., within the cage. Cage cleaning is carried out at a cage-cleaning station (see **Chapter 6.2.1**) so that there is no cross-contamination between animals and the environment.

Figure 5.6. Individual Ventilated Cage

Source: Tecniplast UK

Figure 5.7. Racks of IVCs

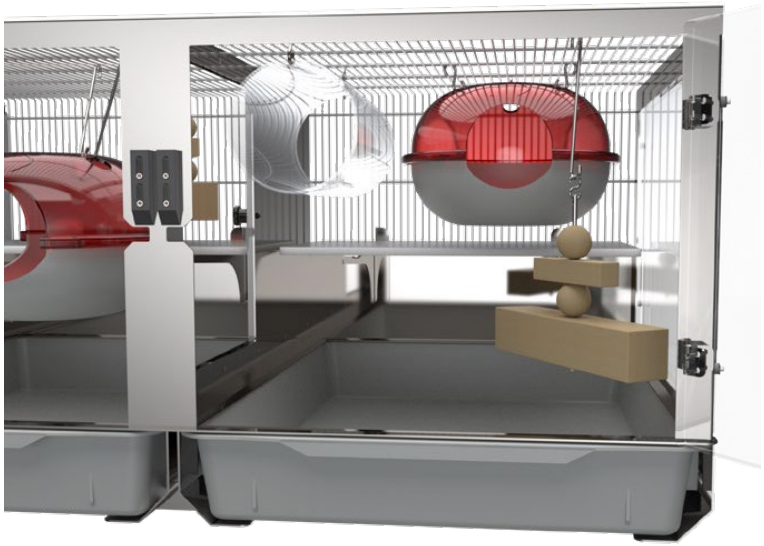
Source: Tecniplast UK

Another development that has benefits in some circumstances involves using disposable caging. As mentioned in **Table 5.1**, cages made with materials such as PET are considered disposable. Instead of cleaning and sterilising cages, the soiled ones are disposed of and are replaced by new ones. Using disposable caging reduces the need for large rooms and equipment for washing cages. Theoretically, the soiled cages can be recycled, but this would mean that they have to be cleaned first, and so defeat one of the main reasons for using them. They are usually incinerated.

Thin liners that sit within IVCs are available. When cages need to be changed, the liners containing the soiled bedding are removed and disposed of. Liners are particularly useful if an experiment involves the use of toxic or infectious materials because they minimise the risk of staff contact.

Another example of a new cage design is linked cages, which give animals a larger and more enriched environment. **Figure 5.8** shows linked rat cages with items for environmental enrichment. Similar cages are available for rabbits and guinea pigs.

Figure 5.8. Linked rat cage



Source: Scanbur

5.4.1. Arrangement of cages

Most small animal cages and racks are integral units. IVC racks hold the air-handling apparatus, and the air inlet and extract ducts must form a firm seal with each cage.

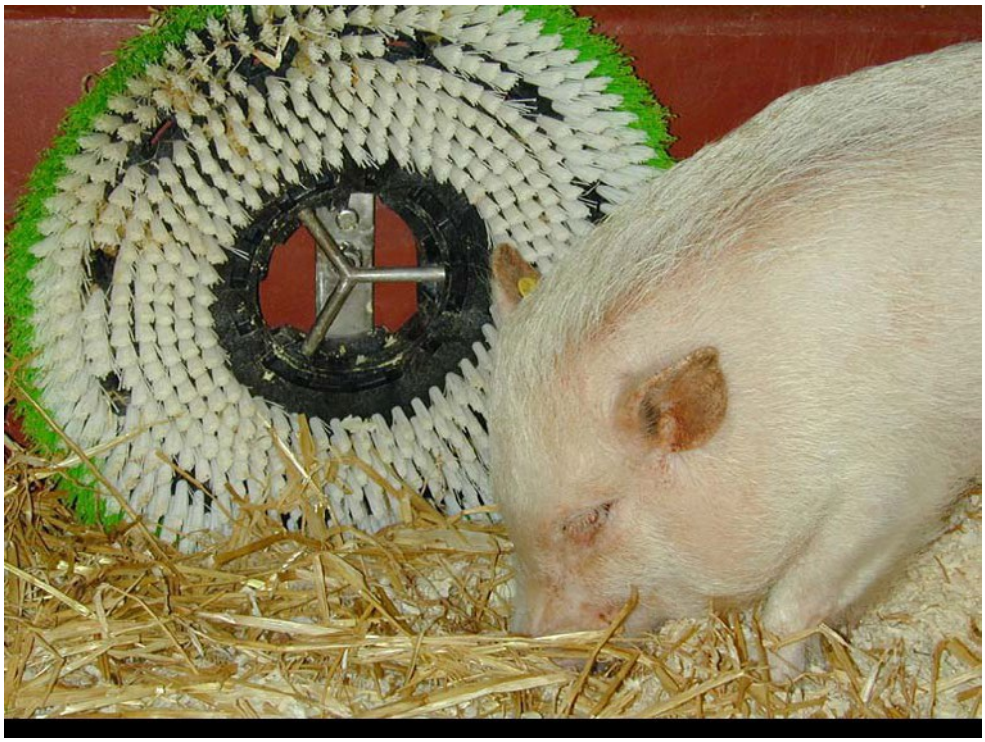
Racks are wheeled so they can be moved to clean around them and to move them to and from the cage wash area. Cages can also be arranged in batteries, whereby banks of cages are connected to one another (e.g. chicken batteries).

5.4.2. Pens

Floor pens are used for larger species such as dogs and pigs; they allow more space for the animals, and therefore a greater opportunity to fulfil their behavioural needs.

Pigs are very strong creatures and the pen structure has to be equally strong to prevent them from destroying it (**Figure 5.9**). Dogs are less destructive but still need a solidly designed pen to hold them. Rabbits and guinea pigs can also be housed in floor pens (**Figure 5.10**).

Figure 5.9. Pig pen with scratching brush as environmental enrichment



Source: Pfizer UK

Figure 5.10. Rabbit floor pen

Source: Sarah Lane

5.5. Example of a non-mammalian species Zebrafish

Zebrafish tanks are available in various sizes and are held within either a multi-rack system (**Figure 5.11**) or a stand-alone system (**Figure 5.12**). The flow of water provided to these racks can be through a recirculating water system (water is filtered and reused) or a flow-through system (water flows direct to the drain upon exit of the tank). The most commonly used method is the recirculating system; this allows water to enter the tank via the lid and then circulate within the tank before exiting through a baffle to the filtration system. The filtration system consists of a four-stage process: mechanical filtration (removal of solid wastes), biological filtration (oxidisation of ammonia, nitrite, nitrate), chemical filtration (removal of chemicals such as chlorine) and sterilisation (reducing the spread of pathogens present in water).

Stocking density per tank can vary between facilities, and 5–8 adult fish per litre is used as a guide. From December 2026, in line with Annex III of the Directive, the stocking density of zebrafish cannot exceed 10 adult fish per litre, and water volumes smaller than 1 litre can no longer be used for adult zebrafish in the EU. Young fry can be housed at much higher densities of 50 per litre due to their small size and their creation of less waste than adults.

Figure 5.11. Multi-rack system showing several connected racks for Zebrafish

Source: Nicola Goodwin

Figure 5.12. Stand-alone system for Zebrafish

Source: Nicola Goodwin

5.6. Environment

The environment of an animal is made up of everything that surrounds or exerts an effect on it. The conditions in close contact, bounded by the enclosure, are referred to as the micro-environment. The term 'macro-environment' is used when referring to conditions outside the micro-environment.

A great deal of effort is dedicated to establishing and maintaining a suitable environment to house laboratory animals; this is important for several reasons. The first is the obvious one of ensuring the animals' health and welfare. Captive animals have very limited opportunity to control their own environment, so it is the responsibility of their carers to do it for them.

The second reason for maintaining a stable environment is to reduce variation in experimental results. Variation in the environmental levels can affect the way in which animals respond to experimental procedures; to obtain reliable and reproducible results, the environment must be kept stable.

It is also important to note that people work in animal facilities and the environment must be comfortable and safe for them too.

Individual animal species differ in the way they respond to their environments, and there are major differences between the way animals and humans respond to the same environment. For example, for most mammals, smell is a much more important sense than it is for humans, and they can gain a lot of information from scents produced by another animal, even if that animal is no longer present. Many laboratory animal species are able to hear sounds at a much higher frequency (pitch) than humans. These and other differences must be considered when establishing and maintaining a suitable environment for animals.

Many research projects have been carried out, and continue to be carried out, in an attempt to establish environments that allow different species to thrive in laboratory conditions. The results of those studies form the basis of the standards used today. The legal requirements on the care and

accommodation of the animals used for scientific purposes can be found in Annex III of the Directive, and further recommendations can be found in Commission Recommendation 2007/526/EC.

Many factors make up the environment of laboratory animals. They include the following:

— cage, bedding, diet, water (see **Chapter 7**), other animals,

— temperature, relative humidity, day length, light intensity, noise,

— air (the oxygen, carbon dioxide, water vapour, ammonia and dust contents),

— routine care procedures (see **Chapter 6**),

— human interactions,

— breeding systems (see **Chapter 8** and **Chapter 9**),

— invasive procedures (see **Chapter 11**),

— microorganisms, diseases and treatments (see **Chapter 4** and **Chapter 6**).

Some of these factors are easy to control, others much less so. The factors listed above that are not discussed in detail in this chapter are covered elsewhere in this publication.

5.6.1. Other animals

Where conspecifics (animals of the same species) are group-housed, enclosure mates form an obvious and important part of the micro-environment. They communicate, groom each other, play, fight, establish hierarchies, etc. Conspecifics occupying other cages or pens influence the environment of other occupants of the room by visual, aural and olfactory means.

Most laboratory species are sociable and should be housed in groups unless there are welfare or experimental reasons for them to be housed alone. Separation for experimental reasons should be done only after careful consideration and discussion with those responsible for the care and welfare of the animals. They should be separated for the shortest time possible and they should be in sight, sound and smell of their conspecifics. Extra contact with care staff could also be beneficial to animals that are housed singly. Dependent on the duration and frequency of single housing for scientific reasons, an exemption from standard housing would need to be requested as part of project application process.

Some species, such as hamsters, are not social and, unless housed together at weaning, will fight, so single housing for adults may be necessary. This is especially the case if the animals have not been brought up together.

Even among animals considered socially compatible, there are strains that are more aggressive, particularly males. Mice are generally considered to be social animals, and if they are housed singly they show signs of distress. These signs do not appear when they are group-housed. When new groups are set up, some aggression may be seen, but usually this is just the group working out a hierarchy, which is rapidly established and the animals quickly settle down. Action should be taken if disturbance or fighting is prolonged. Problems that may occur in setting up new groups can be minimised if the grouping is done when the animals are newly weaned, they are put in an enclosure new to all of them and there is plenty of nesting material and environmental enrichment items where an individual can hide if it is being bullied. Once a group has been established, animals should not be removed (unless it is for welfare reasons) because it may upset the hierarchy and a new bout of fighting may occur.

New animals should not be added, as the established group may attack the newcomer. NC3Rs has published a guidance paper, 'Minimising aggression in group-housed male mice' (NC3Rs 2024).

Mixing different species in the same room should be avoided whenever possible. Predator and prey species should never be housed in the same room; in fact, they should not be housed in sight, sound or smell of each other. The same is true of animals that are often incompatible, such as cats and dogs. Noisy species should be housed away from quieter ones.

Overcrowding in cages and pens causes stress and leads to increased incidence of ill health and fighting.

5.6.2. Heating, ventilation and air conditioning system (HVAC)

In order to provide a stable environment, help control disease outbreaks, maintain breeding productivity and increase security, the majority of laboratory animal facilities do not have windows that open. Filtered air at the required temperature and humidity, free from noxious gases and dust, is supplied, and contaminated waste air removed, through a heating, ventilation and air conditioning system (HVAC).

A typical air-handling system takes the air from outside and passes it through several air filters of decreasing fineness. The final one is called a High Efficiency Particulate Air (HEPA) filter, which removes up to 99.97% of the particles in the air. The system adjusts the temperature and humidity and distributes it around the facility. Before entering the animal room, the air passes through secondary heaters and humidity regulators so that the temperature and humidity can be adjusted for individual species. Sensors in the room measure the temperature and humidity and feed the results back to a control centre so that adjustments can be made to keep the levels stable.

Air extracted from the room is ducted to the outside and is not recirculated. However, in modern air-conditioning systems, heat is recovered from the extracted air to help warm the air entering, and so save energy. Rough pre-filters are attached to room extract ducts to prevent large amounts of dust and dander (fur) from entering the ducting and, in the event of a system failure, to prevent potentially contaminated air in the ductwork from leaking back into the animal room.

Treated air has to be supplied to animal rooms so that all areas of the room are covered and all animals are supplied with air of the desired quality, while also being protected from draughts.

The rate of supply and extraction of air can be adjusted so that rooms can be at positive or negative pressure. In a room housing animals in open cages, if air is supplied at a faster rate than it is extracted, the room will be at positive pressure. This means that pathogenic agents and other airborne matter cannot enter the room through gaps around the door or when the door is opened. If, however, the research in the room involves work with infectious organisms or other harmful material, the main focus must be on preventing escape from the room, so air is supplied at a lower rate than it is extracted, resulting in negative pressure in the room.

If animals are housed in IVCs, the air can be supplied at positive or negative pressure directly to the cage (see **Chapter 5.4**).

Air pressure gauges (called manometers or a Magnehelic®) measure the air pressure in a room, compared with the corridor outside (**Figure 5.13**).

Figure 5.13. A Magnehelic pressure gauge, which shows that air pressure in the room is slightly higher than that in the corridor

Source: Stephen Barnett

Where facilities use potentially harmful organisms, the air that is extracted from the room must be treated and/ or filtered before it is released into the atmosphere.

The amount of fresh air supplied to a room in order to provide the animals with their needs is measured in air changes per hour (ACH). The number of air changes per hour required depends on the species being housed and the stocking density. The guidance given in Commission Recommendation 2007/526/EC is that 15–20 ACH is normally adequate for a fully stocked room of rodents or rabbits in conventional housing, dropping to 8–10 ACH if the stocking density is low. In rooms housing rodents in IVCs, cats, dogs and non-human primates, 10–12 ACH may be sufficient. In IVCs themselves, where air is directly supplied to and extracted from the cage, air changes of 50 or 75 ACH are provided, depending on the design of the cage and the stocking density. Sensors connected to alarms are fitted to warn of system failures. It is essential that problems with air supply are dealt with quickly; in a sealed cage, the welfare of the animals will deteriorate very rapidly if air supply is compromised.

A good indication of whether conditions in the room are suitable is apparent when the room is entered. If the temperature and humidity are within acceptable limits and there is no build-up of ammonia or other noxious odours, the conditions will feel comfortable. If there is a high level of ammonia, it could mean that the air changes are insufficient, but it could also mean that the room is overstocked or the cleaning routine is inadequate. The cause must be identified and rectified.

Ventilation systems can be a source of noise and vibration that will disturb the animals. To minimise this disturbance, the main air-handling plant should be situated as far away from animals as is practicable and the system of motors and fans must be balanced to minimise the audible sound and ultrasound being emitted.

Farm animals used for scientific purposes benefit from being kept outdoors or in housing ventilated with natural air such as the weatherboarding shown in **Figure 5.14**. Any animal housed outside should have access to shelter from wind and rain. They should not be restricted to outdoor areas under climatic conditions that will cause them distress.

Figure 5.14. Farm animal housing showing a solid wall base topped with weatherboarding. This arrangement allows an adequate airflow above the animals while protecting them from draughts

Source: Stephen Barnett

5.6.3. Temperature

Animals are classified as being either ectothermic or endothermic. Reptiles, amphibians, fish and invertebrates are ectotherms, which means that they cannot regulate their body temperature by physiological means, although they are efficient at doing it by behavioural means (e.g. basking in the sun to warm (**Figure 5.15**) or seeking shade to cool).

Figure 5.15. Agama lizard basking in the morning sun

Source: Stephen Barnett

Mammals and birds are endotherms. They can regulate their body temperature within a narrow range by physiological and behavioural means, so, if the ambient temperature increases or decreases, their internal body temperature remains constant.

If the ambient temperature drops, body heat can be conserved by constricting surface blood capillaries (lessening the loss of heat from the blood), piloerection (raising the body hair to trap a layer of insulating air), sitting hunched up (lessening the amount of body surface exposed to the cold outside air), nest building and sitting close to cage mates. Shivering helps because heat is produced as a by-product of the release of energy used to cause the muscles to move.

If ambient temperature rises, body temperature can be kept stable by dilating surface blood capillaries (bringing more blood to the surface and increasing the loss of heat from the blood), panting and/or sweating (due to the latent heat of vaporisation) and lying spread out to expose the maximum surface area of the body from which heat can be lost.

The temperature regulation mechanisms of endotherms work over a fixed range of ambient temperatures. Outside this range, endotherms will suffer from heat and cold stress. If the extreme temperatures go on for long, they can prove fatal. Small animals such as rodents have a fast metabolic rate that makes them much more sensitive to temperature changes. Larger species such as dogs and cats can tolerate a wider range of ambient temperatures.

The mechanisms used to maintain a stable body temperature (called thermoregulation) are perfectly normal physiological responses, but they affect the metabolism and behaviour of animals, which can influence the results obtained from a range of experiments. For this reason, in addition to providing for the comfort of the animals, it is important to select a suitable temperature in which to keep them and to make sure, once it has been selected, that it does not vary more than ± 2°C. **Table 5.2** gives the temperature ranges for various species, as stated in Commission Recommendation 2007/526/EC. These figures are suitable for adult, healthy animals and would need to be adjusted for young, hairless or otherwise compromised animals. It is necessary to ensure that the temperature in a holding room is adapted to the species and age of the animals housed. There is a mandatory requirement to measure and record room temperature on a daily basis.

Farm animals housed outside can tolerate very cold weather, particularly if they are outside all the time and grow thick coats in winter, but no animal can survive long periods exposed to wind or draughts. They must be provided with access to shelter from both wet and wind. Some Old World non-human primate units give the animals access to outside pens. The animals appear to benefit from this, but they must have access to shelter and to heated inside accommodation when needed.

Table 5.2. Suitable temperature ranges for adult uncompromised animals

Animals	Temperature range (°C)
Mice, rats, gerbils, hamsters	20-24[3]
Guinea pigs	15-21
Rabbits	15-21
Cats	15-21
Dogs	15-24
Ferrets	15-24
Marmosets and tamarins	23-28

Source: Commission Recommendation 2007/526/EC.

[3] At the lower end of this range, it is recommended that small rodents should be provided with nesting material for extra warmth if needed.

5.6.4. Humidity

Humidity is the amount of water vapour in the air. Relative humidity (RH) **is the amount of water vapour contained in a volume of air expressed as a percentage of the water vapour that would be in the same volume of air if it were saturated and at the same temperature** (saturated means that it can support no more water). The amount of water vapour present in air is dependent on its temperature. Warm air can support more moisture than cool air, so, if the temperature rises, the RH will fall, even though the amount of water in the air remains the same. The opposite is also true; cold air can support less water vapour than warm air, which explains why moisture in the air in a warm room condenses on cold windows. It can be seen from this that temperature and humidity are closely linked.

As with all environmental factors, wide variations in humidity or long periods at either a low or a high level of humidity will stress some animals and may create the conditions in which disease can develop; for example, low humidity, particularly if it is with high temperature, may cause a condition known as ringtail in rats and mice (**Figure 5.16**), and encourages some types of respiratory infections. High humidity can lead to facial dermatitis in gerbils. High humidity can provide conditions in which moulds will grow. High humidity also speeds up the release of ammonia from urine and faeces, which, in turn, can lead to respiratory disease.

Other effects of varying the levels of temperature and humidity are summarised in **Table 5.3**.

Figure 5.16. Ringtail in rats

Source: Source Newcastle University

Rodents, especially mice, rats and gerbils, are more sensitive to variations in humidity than larger species, such as dogs and cats. The humidity levels given in Commission Recommendation 2007/526/EC are shown in **Table 5.4**.

Table 5.3. Examples of the effects of temperature and RH variation on experimental results

Changes in food and water intake	The amount of food and water an animal consumes is affected by the ambient temperature and humidity; for example, if the temperature is high and the humidity low, they will drink more. If test substances are added to food or water, variation in temperature and RH will affect the dose they receive
Changes in drug activity	Variations in temperature and RH can increase or decrease the toxicity of drugs e.g. a 4°C increase in ambient temperature has caused a tenfold increase in toxicity with one drug
Increase/decrease in fertility, lactation and teratogenesis (congenital abnormalities)	Repeated short exposures to extremes can have cumulative effects, particularly on the development of the nervous system of some species
Incidence of disease	This may be direct (e.g. incidence of ringtail in rats related to low RH levels; **Figure 5.16**) or indirect and related to bacterial survival and activity (e.g. ammonia production and its relevance to the induction of respiratory disease)

Source: Clough (2007)

Table 5.4. Suggested RH (%) for laboratory animals

Animals	RH (%)
Rodents (except gerbils)	46-65
Gerbils	35-55
Rabbits	Not less than 45
Cats	No need to control
Dogs	No need to control
Ferrets	No need to control
Non-human primates	40-70

Source: Commission Recommendation 2007/526/EC

5.6.5. Light

Most laboratory animals are kept under artificial light. Artificial light can be controlled to suit animal husbandry and experimental needs. However, Commission Recommendation 2007/526/EC advises that the provision of non-opening windows should be considered in holding rooms because natural light provides environmental enrichment for some species, for example non-human primates, dogs, cats, farm animals and other large mammals (**Figure 5.17**).

The light environment is made up of three elements: intensity, photoperiod and wavelength. All of them have the potential to affect animal welfare, breeding performance and experimental results if not adjusted correctly.

Figure 5.17. Dog unit fitted with glass bricks to let in daylight while maintaining security

Source: Cube Cleantech Ltd

Intensity

Light intensity (or brightness) is measured in units of lux. To give an idea of what lux is, the brightness of full sun can produce a reading of about 100 000 lux, whereas full moonlight gives less than 20 lux. The light levels in an animal room should be sufficient to ensure that the animals can be inspected and that husbandry can be carried out properly. Levels of 350–400 lux measured at 1 metre above the ground have been found to be suitable for this purpose. Light meters can be used to determine the light levels (**Figure 5.18**). Light intensity within a room will vary due to the position of the light source and the distance from the source. Near the light source, the reading may be as high as 700 lux, whereas, in a cage at the bottom of a rodent rack, it could be as low as 10 lux. Low light levels in cages and pens are beneficial for rodents.

Figure 5.18. Light meter reading 419 lux

Source: Stephen Barnett

Many laboratory species are nocturnal (active at night) or crepuscular (active at dawn and dusk), so their eyes have adapted to function in dim light. Exposure to bright light can cause retinal damage. The situation is made worse if the animals are albino, as they have no protective pigment in their

eyes (**Figure 5.19**). Retinal damage has been seen to occur in rodents housed at the top of a rack nearer a light source; for this reason, the top of rodent racks must be shaded from light.

Figure 5.19. Pink eyes of an albino rat, indicating lack of retinal pigment

Source: Stephen Barnett

Some laboratory animal facilities are fitted with a two-level light system: the higher intensity is used to provide staff with adequate light to carry out their duties, and a lower level is used when the husbandry has been completed and the animals are on their own. It has been suggested that varying the light levels to imitate dawn, dusk and moonlight is beneficial for most mammalian species.

Photoperiod

Photoperiod is the length of time for which the animals are exposed to light during the day.

Photoperiod affects several biological processes that vary over 24 hours. These variations are called circadian rhythms (from two Latin words, *circa*, meaning approximately, and *diem*, meaning day). Circadian rhythms, in common with other physiological cycles that vary over time, are controlled by an internal body clock, but they are synchronised by environmental factors, most notably by light. The purpose of circadian rhythms is to use the resources of the body in the most efficient way.

Examples of the circadian rhythms affected by light include sleep, feeding patterns, body temperature, body weight and hormone levels. Variations in photoperiod upset the circadian rhythm and affect breeding and experimental results. Affected hormones include those that control the reproductive cycle.

If kept in natural light, some species will be affected by the seasonal variation in the light/dark cycle. These variations often disappear with a photoperiod of constant length, for example 12 hours of light and 12 hours of dark. The Syrian hamster, for example, will stop breeding when the daylight hours are shorter in winter, but will continue to breed all year if kept in a constant photoperiod of 12 hours of light and 12 hours of dark.

Artificial lighting systems can be adjusted to reverse day and night or half-reverse day and night, if required for experimental purposes.

Continuous darkness has the effect of lengthening the oestrous cycle in many mammals, and continuous light can produce continuous oestrus. Both situations will result in a drop in breeding productivity and eventual cessation of breeding.

Even a short flash of light in the dark phase of the day can affect the circadian rhythms of some animals, which may, in turn, affect experimental and breeding results. If these animals need to be inspected in the dark phase, it is better to use a dim red light rather than to turn on the normal lights, as many species are less sensitive to red light.

Wavelength

Most mammals can perceive colour, but not all have the same type of colour vision. The retina, which lines the back of the eye, contains two major types of light-sensitive receptors, rods and cones. Rods detect light and dark and enable vision in black and white. Cones detect colour, but different cones detect different colours: one type detects blue (a short-wavelength colour), a second type detects red (a long-wavelength colour) and a third detects green (a medium-wavelength colour). Most primates (human and non-human) have all three types of cone in their retinas; all three together allow the perception of a full range of colours, in a system called trichromacy. Most other mammals have only two types of cone (red is missing), so they still see a range of colour, but not the same range as non-human primates.

As has been mentioned previously, daylight is known to be beneficial to a number of species. There is also some experimental evidence to show that a low level of ultraviolet (UV) light has a beneficial effect on all animals housed indoors. Imitation daylight lights that contain a UV element are recommended when animals are kept under artificial lighting.

5.6.6. Noise

Noise is unwanted sound. At high levels it can startle animals, interfere with their communication with conspecifics and cause stress that affects their welfare, breeding and experimental results. It is important, therefore, to keep the noise levels as low as possible. Sound has two properties, intensity and frequency.

Intensity and frequency

Sound intensity (or volume) is measured on a logarithmic scale in decibels (dB) (**Figure 5.20**). The pitch of a sound depends on its frequency: the higher the frequency, the higher the pitch. Frequency is measured in hertz (Hz).

Not all species are sensitive to the same sound frequencies. Humans with good hearing can hear a range of frequencies from 20 Hz to 20 kHz, but are most sensitive to sounds in the range of 0.5–5 kHz. These sensitive frequencies appear to be louder than other frequencies to the human ear, even though the intensity is the same. For this reason, straight dB measurement is not very useful when assessing how annoying a sound is to humans, as it treats all sound the same regardless of its frequency. To overcome this problem, sound meters have been developed that filter sound so that extra consideration is given to the frequencies that humans are most sensitive to. Measurements on this scale are called dB (A-weighted scale), or dBA.

Figure 5.20. Sound meter reading 58.2 dBA

Source: Stephen Barnett

Any frequency below the lower threshold of human hearing (0.2 kHz) is called infrasound. None of the usual laboratory species are known to communicate in this range, but elephants, rhinoceros and hippopotamuses are examples of animals that do. Frequencies above the threshold of human hearing (20 kHz) are called ultrasound. Many laboratory species can hear in ultrasound, for instance rodents, rabbits, cats, marmosets and tamarins. As an example, rats have a hearing range of 0.25–76 kHz and are most sensitive between 35 kHz and 40 kHz, and cats can perceive sound in a range from 0.07 kHz to 91 kHz, with a peak sensitivity of 1–40 kHz.

Loud or long-lasting noise, noise of a frequency that is annoying and sudden irregular noise will cause stress, compromise welfare and give rise to changes in behaviour, a drop in breeding performance and unexpected experimental results. In susceptible strains of mice, some noise may cause audiogenic seizure (fits).

Sounds produced by animals form an important element in their communication repertoire; for instance, it is important during courtship, mothering, excitement, aggression and defence. High ambient noise levels will interfere with this communication.

It follows that it is necessary to control noise levels to make sure that they remain within acceptable limits. The starting point in reducing noise is to identify the sources in animal facilities, as detailed below.

— Animals themselves: most species make some noise, but usually it is at a fairly low level and does not cause problems. However, some (e.g. dogs, non-human primates and pigs) produce sound that often exceeds 90 dBA, a level that will cause permanent hearing loss in humans. Rooms holding these animals should be fitted with sound insulation and sound-absorbing materials. Quiet animal species should be housed away from noisy ones so that they will not be disturbed by them. Humans must wear ear protectors when entering rooms of noisy animals (e.g. beagles), particularly at feeding time or exercise periods.

— Apparatus: traditional metal cages, bins, etc., have given way to modern plastic or composite versions, which has significantly reduced day-to-day noise production in animal facilities. Human activity can still generate noise, so staff have to be aware of this and work as quietly as possible. Silentone™ fire alarms produce sound at a frequency that humans can hear but that the more sensitive animals cannot. Commission Recommendation 2007/526/EC states that these should be fitted in animal facilities as long as they do not compromise human safety.

— Routines: it is impossible not to generate noise with some animal facility activities, for instance cage cleaning and loading and unloading goods. These activities should be carried out as far away from animals as possible. Controlling noise that humans can hear is usually straightforward; it is more difficult with ultrasound. Ultrasound is generated by a number of common activities, for example filling water bottles or using hoses, vacuum cleaners, visual display unit screens or trolley wheels. The effect of some of these activities can be mitigated; for instance, plastic sinks can lessen the production of ultrasound, and water guns are quicker, quieter and more hygienic than taps.

Commission Recommendation 2007/526/EC advises that the acoustic environment is monitored over a broad range of frequencies. Sound pressure meters give an overall noise measurement (dBA), and ultrasound detectors (bat detectors) can help detect sources of ultrasound in an animal room, but more sophisticated equipment (an integrating octave-band sound-level meter) and operators are required to monitor rooms over a broad range of frequencies.

5.6.7. Vibration

Most animals are sensitive to vibration; like noise, it causes stress. In addition to the welfare consideration, it has an adverse effect on reproduction and experimental results. Animals must be housed away from sources of vibration, such as cage wash areas and lifts. Although external sources of vibration and noise, for example building works, are outside the control of those managing laboratory animal facilities, the effect they have on the welfare of animals and experimental results must be noted.

5.7. Example of a non-mammalian species Zebrafish

Notes on the environmental needs of zebrafish, by Nicola Goodwin

Water provided to the fish tanks should be treated to remove impurities. A reverse osmosis process can be used for this, allowing only pure water to pass through a membrane before entering the aquatics system.

The water in which zebrafish are housed must be conditioned to ensure optimal welfare; once conditioned, this water can be referred to as ‘system water’. The key environmental conditions for system water are pH, salinity/ conductivity, dissolved oxygen and temperature. All of these parameters must be checked daily and adjusted as required to ensure optimal health and welfare.

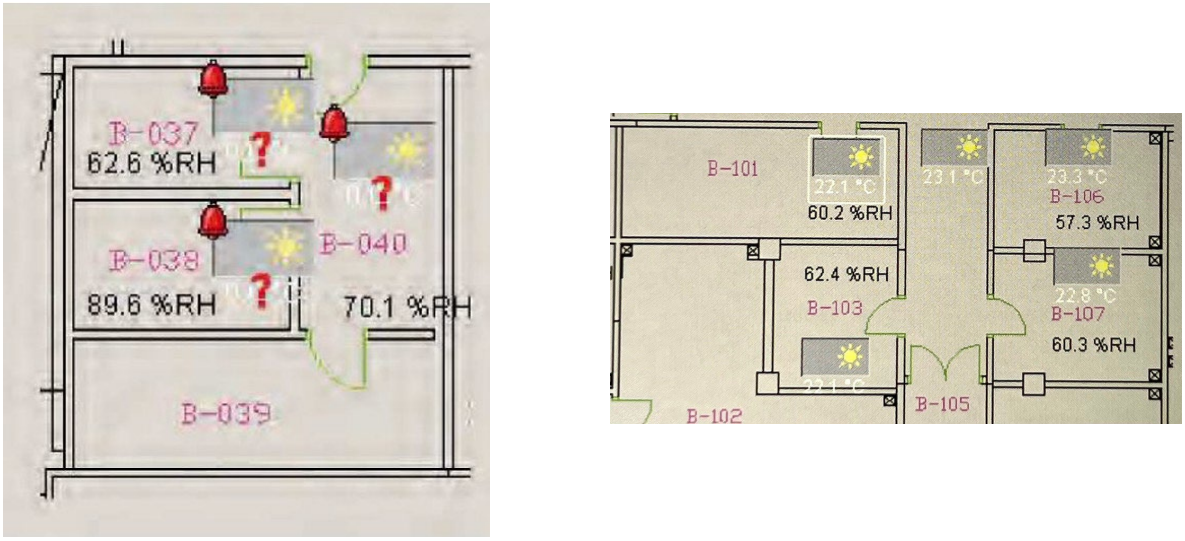
From December 2026, in line with Annex III of the Directive, these parameters should be within the following limits: pH, 6.5–8.0; conductivity, 150–1 700 μs/cm^2; dissolved oxygen, more than 5 mg/l; and temperature, 24–29°C.

5.8. Monitoring the environment

The animal facility environment must be monitored to ensure that the systems are working properly. Annex III of the Directive states that temperature must be read and recorded daily, and that other aspects of the environment should be monitored regularly to ensure that animal welfare is maintained and that no environmental variations occur that could compromise experimental or breeding results.

Most laboratory animal facilities are fitted with a building management system (BMS). This is a computer-controlled system that delivers and monitors the required environmental levels. The monitoring system consists of electronic sensors, arranged in the room, which continually collect information on temperature, humidity, etc., and pass it to a computer. The computer not only keeps a continuous record of these values, but also gives warnings if any value falls outside a preset limit (**Figure 5.21**).

Figure 5.21. BMS screen.
Right: a section of the BMS screen showing environmental levels for animal rooms.
Left: a small section of a BMS screen showing warning of some environmental readings outside set levels



Source: Stephen Barnett

Even though computerised monitoring systems are almost universally used in animal facilities, independent instruments that are situated in the rooms still have an important role to play in ensuring that the environment is stable. They are essential if the computerised system fails, and they can be used to check the accuracy of the computerised system. They also give the people servicing the room immediate information on the levels within it.

A monitoring system used within a room should be sited in a position that is most likely to record the general conditions of the room, which means away from the influence of sources of heat, cold and moisture, for example air ducts, doors and sinks.

5.8.1. Measuring temperature and humidity

Temperature is measured in degrees Celsius (°C) and Relative Humidity (RH) is measured in percentages (%). An instrument that measures both is called a thermo-hygrograph (**Figure 5.22**).

Electronic versions of these instruments record the maximum and minimum temperatures in the room since the instrument was last read and reset, as well as the current room temperature. They also give a display the RH level at the time of reading (**Figure 5.23**). These readings must be manually recorded.

Figure 5.22. Thermo-hygrograph

Source: Stephen Barnett

Figure 5.23. Small Electronic Thermo-hygrograph

Source: Gareth Gough, Bristol University

A traditional thermo-hygrograph (as shown in **Figure 5.22**) keeps a permanent record of the variations in temperature and humidity over a week. In these instruments, the temperature sensor is a bimetallic strip arranged in a coil; when the temperature rises, the metal expands and the coil opens, when the temperature falls the coil contracts. The humidity sensor is horse hair, which expands or contracts depending on how much moisture is in the air. These sensors are fixed at one end and attached to levers at the other. Attached to each lever is a pen (one for temperature and one for

humidity); these are both in contact with a sheet of ruled paper wrapped around a drum. The drum is turned by clockwork and takes seven days to do a complete revolution. As the drum turns, the pens record the changes in temperature and humidity. The paper is ruled in hours and days on the horizontal axis and in temperature and humidity on the vertical axis.

Continuous temperature and humidity monitoring can also be carried out using data loggers. The advantage of these electronic devices is that the recordings can be downloaded onto a computer for detailed analysis.

References

Clough, G. (2007), 'The environment', in: Barnett, S. W. (ed.), *Manual of Animal Technology*, Blackwell Publishing, Oxford.

Commission Recommendation 2007/526/EC of 18 June 2007 on guidelines for the accommodation and care of animals used for experimental and other scientific purposes (notified under document number C(2007) 2525), OJ L 197, 30.7.2007, p. 1, ELI: https://eur-lex.europa.eu/eli/reco/2007/526/oj.

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, OJ L 276, 20.10.2010, p. 33, ELI: https://eur-lex.europa.eu/eli/dir/2010/63/oj/eng.

NC3Rs (2024), 'Minimising aggression in group-housed male mice', NC3Rs website, https://nc3rs.org.uk/3rs-resource-library/minimising-aggression-group-housed-male-mice.

Contribution of this chapter to the Three Rs

Both housing and environment contribute to the refinement element of the Three Rs.

This chapter seeks to convey the importance of well-designed enclosures to the well-being of animals. It explains the necessity of providing space, but also urges readers to consider what goes into that space, and describes how consideration of the behavioural needs of animals can inform enclosure design and furniture/material.

The chapter goes on to explain how variations in the environmental levels can affect the physiology of animals, resulting in distress and affecting breeding performance and experimental results. The environmental levels, therefore, must be controlled and monitored.

Self-assessment questions

1. Adequate space is essential in an enclosure, but what else needs to be considered to make the housing suitable for the intended occupant?
2. List three criteria that must be provided in an animal enclosure.
3. Define the term 'micro-environment'.
4. What are circadian rhythms and why is it important to be aware of them in laboratory animal facilities?
5. What is ultrasound and why is it important to control it in an animal facility?

6. Routines, husbandry and hygiene

After reading this chapter, you should be able to describe the routines and husbandry practices for the maintenance, care and welfare of animals used in research (learning outcome 4.1).

6.1. Introduction

Husbandry practices are concerned with the day-to-day care of animals. Carried out properly, they ensure that animals always have access to food and water, are able to clean and groom themselves, have a suitable stable environment, are protected from the risk of disease and are kept under daily observation so that any welfare problems that may arise are noticed and remedied as soon as possible. It is the responsibility of animal care staff to carry out husbandry tasks in a professional and conscientious manner; in doing so, they make a major contribution to the culture of care in an institution.

A routine is a systematic method of completing a husbandry task or series of tasks. Routines should be prepared for all aspects of the husbandry of laboratory animals; they enable efficient planning, ensure that nothing is missed and ensure that all staff carry out the work to the same preset standards. This is good for the animals because they become accustomed to the routine and are therefore less concerned at the disturbance. It also benefits the scientific studies because variations in the way that animals are cared for may affect experimental results.

6.1.1. Routines

Basic routines are the same for all animals and all types of facility. They include:

— checking and recording the environment (temperature, humidity, air pressure) (see **Chapter 5.6**);

— checking the physical condition of the animals and carrying out study observations (see **Chapter 4.5**);

— feeding and watering (see **Chapter 7**);

— cleaning pens, cages, trays, etc. (see **Chapter 6.2**):

- changing cages,
- cleaning equipment,
- cleaning the room and its fittings;
- checking cages and equipment for damage;

— record-keeping;

— health and safety procedures (see **Chapter 14**).

Although the basic elements of an animal facility routine are common to all species, they must be adapted to fit the particular needs of each species, method of housing and type of research.

Factors that cause variation in the routines include:

— species and behaviour (e.g. dogs produce wet and smelly faeces, which need to be cleaned out at least once a day; mouse faeces are relatively dry, and so need to be cleaned out less often);

— type of diet (e.g. dry pellets can stay in a hopper for several days; wet food or fresh food must be changed every day);

— cage stocking density and type of work (e.g. a breeding unit routine will differ from a holding room routine by the addition of tasks such as setting up animals for mating, removing young when they reach weaning age, keeping breeding records etc.);

— type of housing (e.g. servicing an IVC is more involved than servicing an open conventional cage);

— study demands;

— exercise/training (e.g. regular exercise for dogs, time in exercise pens or cages for other species and training for experimental procedures).

6.1.2. Standard operating procedures

Once a routine is established, it is written down in detail and staff are required to read and adhere to it. The written routines are often called standard operating procedures (SOPs). SOPs are used by all types of facility, but are a regulatory requirement for studies covered by Good Laboratory Practice (GLP) Regulation. Studies that lead to the licensing of pharmaceuticals or medical appliances must conform to GLP.

Below are examples of SOPs for servicing a mouse room, a rabbit room and a dog room. SOPs cover not only animal rooms but all aspects of work in an animal facility, for example animal handling, record-keeping, health and safety procedures, food-store handling, cage wash procedures and servicing equipment.

The examples below are here as illustrations of the detail that needs to be included in an SOP or routine, and show how routines are put together to ensure that animals and staff are well cared for.

6.1.2.1. Example of a daily-routine SOP for a mouse IVC room

This SOP covers the basic responsibilities when entering a mouse animal room first in the morning and in the afternoon at the end of the working day, to ensure that animal husbandry and welfare standards are being maintained.

Health and safety

Staff must be competent to handle the animals before working with them, to reduce the risk of injury to themselves and the animals in their care.

To reduce the risk of exposure to LAAs and to protect the health status of the animals, PPE must be worn, coupled with local exhaust ventilation systems.

The PPE worn will be prescribed by and dependent on the local conditions, including the health status of the animals. In addition to the normal change of clothing, including mob cap and footwear, the following may be required:

— respiratory face mask,

— overshoes,

— gown,

— gloves.

Supplies/equipment

This will depend very much on the local housing conditions but, for mice housed in IVCs, the following will be required:

— room record book or computerised software system,

— drinking-water supply (autoclaved or filtered by reverse osmosis),

— diet (irradiated or autoclaved),

— bedding (irradiated or autoclaved),

— environmental enrichment (irradiated or autoclaved),

— disinfectant for gloves,

— disinfectant for hard surfaces,

— IVCs,

— IVC change station,

— IVC air-handling system.

General duties

Animal care staff are responsible for the husbandry and health of the animals in the room.

The performance of each task must be recorded and any concerns noted and brought to the attention of senior staff.

Check to ensure that all the IVCs are correctly connected to the racking air supply and that automatic watering, if used, is functioning correctly.

Switch on the cage station and ensure it is working correctly (note that many have alarms) and that it is clean and ready for use.

The local room temperature and humidity should also be recorded, even if there is a BMS.

Ideally, all morning duties should be completed as early as possible so that, if there are animal welfare or other concerns, action can be taken, including reporting to the line manager.

General animal welfare check

Working in an organised and methodical way helps to ensure that nothing is missed.

Beginning at the top row of the first rack, visually check the cages from left to right, and work downwards until all components of all cages on the rack have been checked, including water bottles, if being used. Avoid removing the IVCs from the rack, as this causes stress to the mice.

If the mice are undergoing experimental procedures or breeding, this will influence what observations or husbandry factors need to be actioned and recorded.

Husbandry duties

Ensure that the following is in place for all cages:

— sufficient drinking water is available; if using water bottles, ensure that sufficient water is available until the next fill,

— sufficient diet,

— adequate bedding in the cage,

— environmental enrichment, including nesting material, is present.

If changing the IVC bases, local SOPs should be followed. The frequency does vary, but it should not be necessary to change a mouse IVC more than once a week if the cage has the correct number of mice within.

Afternoon duties should include all the general and animal welfare checks listed above.

In addition, the room should be cleaned, including the cage change station, which should have the hatch fitted and the UV light switched on. The floor should be vacuum-cleaned (not swept, to reduce exposure to LAAs) and mopped with a prescribed disinfectant.

Record that the afternoon duties have been completed.

The area manager or the person responsible for the welfare and care of the animals is responsible for ensuring that all the above duties are carried out in a timely manner and to the correct standard. Any deviation must be recorded and, if necessary, remedial action taken.

6.1.2.2. Example of a daily-routine SOP for a rabbit room

This SOP covers the basic responsibilities when entering a rabbit animal room first in the morning and in the afternoon at the end of the working day, to ensure that animal husbandry and welfare standards are being maintained.

Health and safety

Staff must be competent to handle the animals before working with them, to reduce the risk of injury to themselves and the animals in their care.

To reduce the risk of exposure to LAAs and to protect the health status of the animals, PPE must be worn, coupled with local exhaust ventilation systems.

The PPE worn will be prescribed by and dependent on the local conditions, including the health status of the animals. In addition to the normal change of clothing, including mob cap and footwear, the following may be required:

— respiratory face mask,

— overshoes,

— gown,

— gloves.

Supplies/equipment

This will depend very much on the local housing conditions but, for rabbits housed in IVCs, the following will be required:

— room record book or computerised software system,

— drinking-water supply, bottled or automatic,

— diet pellets,

— bedding, which is often hay (irradiated or autoclaved),

— rabbit cages,

— environmental enrichment,

— disinfectant for hard surfaces.

General duties

Animal care staff are responsible for the husbandry and health of the animals in the room. When carrying out their duties, these must be recorded, and any concerns noted.

The local room temperature and humidity should be recorded, even if there is a BMS.

Ideally, all morning duties should be completed as early as possible so that, if there are animal welfare or other concerns, action can be taken, including reporting to the line manager.

General animal welfare check

Working in an organised and methodical way helps to ensure that nothing is missed.

Beginning at the top row of the first rack, visually check the cages from left to right, and work downwards until all aspects of all cages on the rack have been checked, including water consumption, if bottles are being used. If a water bottle appears to be functioning normally but the water has not gone down at all, or has gone down very little or excessively, it could indicate that something is wrong with the rabbit.

Rabbits housed on grids normally pass a large number of faecal pellets daily. A reduced or excessive amount should be investigated, along with any signs of diarrhoea.

Husbandry duties

Rabbits prefer fresh drinking water, so empty and refill each water bottle daily.

Open the cage doors and check that the food hoppers are not soiled and that diet pellets are available. This is also a good time to check that the rabbit is alert and that there are no obvious clinical symptoms, as many rabbits will come to the front of the cage, especially if you are providing hay at this time and they are used to their carer.

The observations or husbandry factors that need to be actioned and recorded will be influenced by whether the rabbits are breeding, stock or undergoing experimental procedures.

When changing rabbit cages, local SOPs should be followed. The frequency does vary, but it should not usually be necessary to change a rabbit cage more than once every two weeks or to empty the litter trays more than twice a week.

Afternoon duties should include all the general duties and animal welfare checks listed above.

In addition, the room should be cleaned. The floor should be vacuum-cleaned (not swept, to reduce exposure to LAAs) and mopped with a prescribed disinfectant.

Record that the afternoon duties have been completed.

Weekly

Depending on the purpose, rabbits should be clinically examined and weighed once a week. All observations should be recorded on each individual rabbit's health records.

The area manager or the person responsible for the welfare and care of the animals is responsible for ensuring that all the above duties are carried out in a timely manner and to the correct standard. Any deviation must be recorded and, if necessary, remedial action taken.

6.1.2.3. Example of a daily-routine SOP for a beagle dog room

This SOP covers the basic responsibilities when entering a beagle dog room first in the morning and in the afternoon at the end of the working day, to ensure that animal husbandry and welfare standards are being maintained.

Health and safety

Staff must be competent to handle the animals before working with them, to reduce the risk of injury to themselves and the animals in their care.

To reduce the risk of exposure to LAAs and to protect the health status of the animals, PPE should be worn, coupled with local exhaust ventilation systems. However, dogs pose less of a risk regarding LAAs than rodent species, so some facilities do not insist on the wearing of face masks, especially as there is evidence that dogs recognise individual carers by their facial features (Mongillo *et al.*, 2017).

The PPE worn will be prescribed by and dependent on the local conditions, including the health status of the animals. In addition to the normal change of clothing, including mob cap and footwear, the following may be required:

— ear defenders,

— gloves.

Supplies/equipment

This will depend very much on the local housing conditions for beagles, which can be housed singly or in groups, depending on their age and sex and the experimental purpose:

— room record book or computerised software system,

— drinking-water supply,

— diet,

— bedding,

— environmental enrichment,

— disinfectant for hard surfaces,

— cleaning equipment (e.g. brooms, mops, scrubbing machines).

General duties

Animal care staff are responsible for the husbandry and health of the animals in the room. When carrying out their duties, these must be recorded, and any concerns noted.

The local room temperature and humidity should also be recorded, even if there is a BMS.

Ideally, all morning duties should be completed as soon as possible so that, if there are animal welfare or other concerns, action can be taken, including reporting to the line manager.

General animal welfare check

Working in an organised and methodical way helps to ensure that nothing is missed.

Walk around all the pens to ensure that all the beagles are alert and behave in their normal individual fashion. However, beagles will be extremely aware of their carers, knowing each individual, so any new carer or unfamiliar individual will not necessarily see their normal behaviour until the dogs become accustomed to them.

If the beagles are undergoing experimental procedures, breeding or stock, this will influence what observations or husbandry factors need to be actioned and recorded.

Husbandry duties

Ensure the following is in place for all pens:

— sufficient drinking water is available; if using water bottles, ensure that sufficient water is available until the next fill,

— sufficient diet,

— adequate bedding in the cage or raised bed,

— environmental enrichment (e.g. Kong toys).

If exercise facilities are available, the beagle should be allowed out of its pen to use them. This provides an opportunity to give the pen a thorough cleaning, removing any urine and faeces, and washing and drying all the surfaces.

The beagle can then be fed, normally a dry pelleted formulated diet, which will often encourage the beagle to return to its pen from the exercise area voluntarily. This is a good time to reward the beagle by stroking and praising it for returning, while taking the opportunity to give it a quick health check.

Afternoon duties should include all the general duties and husbandry and animal welfare checks listed above. Although it may not be necessary to thoroughly wash the pen each day, any urine or faeces should be removed.

Record that the afternoon duties have been completed.

Weekly

Depending on the purpose, beagles should be clinically examined and weighed once a week. All observations should be recorded on each individual beagle's health records.

The area manager or the person responsible for the welfare and care of the animals is responsible for ensuring that all the above duties are carried out in a timely manner and to the correct standard. Any deviation must be recorded and, if necessary, remedial action taken.

6.2. Hygiene

Hygiene is concerned with the maintenance of health. Hygienic conditions are achieved by the use of routine cleaning, sterilisation and disinfection (or sanitation). These techniques reduce the amount of dirt and the number of agents that can cause infectious disease and allergic reactions. They also reduce the noxious odour that could build up, thereby providing a healthy environment for animals and people within the animal facility.

As mentioned in **Chapter 4**, the agents that cause disease are viruses, bacteria, fungi (moulds and yeasts), and invertebrate parasites and their eggs and larvae (e.g. protozoa, worms, insects and arachnids). Of these, bacterial spores are the most difficult to destroy. Bacterial spores are formed by two genera of disease-causing bacteria, *Bacillus* and *Clostridium*, when their environment becomes hostile; they are very resilient and can exist in spore form for very long periods. When the spores find a suitable environment, they begin to grow and reproduce again. Bacterial spores are resistant to many disinfectants, but will be killed by autoclaving. More dangerous agents require special handling and cleaning techniques, which are outside the scope of this book.

6.2.1. Cleaning cages and pens

How often cages are changed and bedding is replenished are determined by many interrelated factors:

- the cage – design (e.g. IVCs need changing less often than open cages), size,
- the animals (numbers of animals in the cage, species, whether stock or breeding, behaviour of the individuals),
- the type of diet and presentation of food and water,
- the amount and type of bedding used,
- experimental requirements,
- disturbance to the animal.

There is always a compromise between these factors. For instance, it is important to provide a clean environment for animals and to ensure that they are not exposed to high levels of ammonia in the cage (which comes from the breakdown of nitrogenous waste in faeces and urine); however, changing cages disturbs the animals, especially small rodents, and they take time to settle down once they have been placed in the new cage.

The first stage in cleaning is to move animals from the dirty cage to a new cage prepared with bedding and nesting materials. It is common practice to transfer some visually unsoiled environmental enrichment items or nesting material from the used cage to the clean one, as these will carry familiar scents, making the new cage less strange to the animals (Meller *et al.*, 2011).

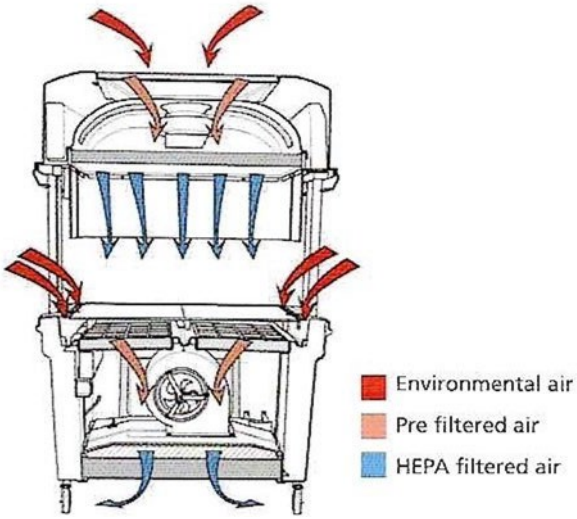
Once animals are removed from the cage, the soiled bedding and nesting materials must be discarded before the cage can be washed. Both removing animals and emptying bedding from a cage cause the release of allergens (see **Chapter 14.4.2**) and other contaminants into the atmosphere, creating a risk to animals and staff. Work practices should be designed to eliminate, as far as possible, these allergens. One way of doing this with small rodents is to use cage-cleaning stations (**Figure 6.1**).

Figure 6.1. Cage-cleaning station

Source: Tecniplast UK

Cages are removed from the rack to the cleaning station and the animals are transferred to clean cage bases; sometimes new lids, food and bottles are used, but it is quite common to transfer the lids, etc., with the animals to the new base. The airflow in the station is designed so that no contaminants from the cages can escape and no unfiltered environmental air can reach the animals. **Figure 6.2** shows the pattern of airflow.

Figure 6.2. Airflow through a cage-cleaning station



Source: Tecniplast UK

It is essential to use cage-cleaning stations when servicing animals housed in IVCs, as the purpose of these systems is to protect animals from the external environment. The cleaning station must be disinfected at regular intervals (e.g. after every fifth cage change) to limit cross-contamination.

In large facilities, robotic (or automated) systems may be used to handle the dirty cages (**Figure 6.3**). Dirty cages are transferred to the cage wash area on trolleys; they are covered to prevent the dispersal of contaminants on the journey to the cage wash (**Figure 6.4**). Robots lift the cage from the trolley, invert it over a waste chute to remove the soil and then place it on the conveyor of a tunnel washer. At the other end of the washer, the clean, dry cage is held under a hopper to receive a measured amount of bedding and then replaced on a trolley to be transported back to an animal room or to the autoclave. With these systems, people can be excluded from the area where there are likely to be high levels of allergens.

Figure 6.3. Robot working in a cage wash area

Source: Tecniplast UK

Figure 6.4. Cover on a trolley carrying dirty cages

Source: Tecniplast UK

Where robotic systems are not available or when pens or larger cages are being cleaned, soiled material has to be removed manually; vacuum extraction systems reduce exposure to airborne allergens.

At all stages in the cage-cleaning process, staff in the area must wear appropriate protective clothing, including face masks or respirators.

6.2.2. Cage wash area

The cage wash area is normally divided into two separate sections, the dirty side and the clean side. The only way cages, racks, etc., can pass from the dirty side to the clean side is through a cage washer. Staff should work in either one area or the other, and should never pass from the dirty side to the clean side without showering and PPE changing process (see **Chapter 4.3.5**).

Cage-washing machines

Three main types of cage-washing machine are available: tunnel washers (**Figure 6.5**), for a high volume of small animal cages; cabinet washers (**Figure 6.6**), for smaller volumes of small animal cages; and rack washers (**Figure 6.7**), for small cages arranged on special racks (**Figure 6.8**), racks themselves, and large cages and other large equipment. The stages of cage washing are the same for all three types (**Table 6.1**). The main difference is that, for the tunnel washer, cages are on a moving track, which carries them through different zones along the tunnel for different treatments, whereas, in rack and cabinet washers, the items being washed stay still and the different washing stages are delivered, in turn, through nozzles in the cabinets.

The stages of the washing process are outlined in **Table 6.1**.

Figure 6.5. Tunnel washer

Source: Tecniplast UK

Figure 6.6. Cabinet washer

Source: Tecniplast UK

Figure 6.7. Rack washer

Source: Tecniplast UK

Figure 6.8. Cages arranged on a rack ready to go into a rack washer

Source: Tecniplast UK

Table 6.1. Stages in the cage-washing process

Stage/zone	Activity
Loading	In robotic systems, the robot places the cages, etc., in the tunnel washer; otherwise, they are loaded by hand. They must be loaded in accordance with the manufacturer's instructions to ensure that they are properly washed
Pre-wash	This stage ensures that any remaining sawdust or other organic matter is removed
Wash	Hot water and a suitable detergent wash the items. The detergents may be acid, alkali or neutral
Neutralising wash	Long-term exposure to acids or alkalis can be harmful to caging materials; the neutralising wash eliminates the risk
Rinse	This stage removes any remaining traces of the chemicals used in the washing process
Drying	The temperature is raised to start the drying process
Unloading	Cages are removed on the clean side of the cage wash area, where they continue to dry, or they can be loaded into an autoclave

Source: Stephen Barnett

Bottle washers

Automated systems are available that remove the caps from bottles, empty the remaining water, wash, refill and re-cap them and then load them onto trolleys so they can be taken back to the animal room or to the autoclave (**Figure 6.9**). These systems not only save time but also limit the risks of repetitive strain injury, caused by removing large numbers of bottle tops, and of back injury, caused by lifting racks of full water bottles.

Figure 6.9. Bottle-cleaning and -filling station

Source: Tecniplast UK

Room cleaning

After the animals are serviced, the room must be cleaned. All work surfaces must be wiped with a cloth dampened with a suitable disinfectant, and the floor must be swept or vacuumed and then mopped with disinfectant. Periodically, the whole room is cleaned. If there has been a disease outbreak, or as part of a routine hygiene programme, the room may be fumigated or fogged (see **Chapter 6.4.1**).

Materials that will come in contact with animals after disinfecting must be thoroughly rinsed with fresh water and dried before the animals have access to them.

6.3. Bedding and nesting

Bedding and nesting materials are necessary to ensure that animals are kept clean and comfortable. Some materials contribute to the enrichment of the animal's environment. The materials also influence the cleaning regime chosen to clean the cage and its surroundings.

Bedding and nesting materials should be obtained from specialist suppliers who ensure that their raw materials come from approved sources and can supply analysis of contaminants, etc., as required for studies conforming to Good Laboratory Practice Regulations.

6.3.1. Characteristics of an ideal bedding material

An ideal bedding material should be:

— Harmless to animals, in other words non-toxic (containing no poisonous material), non-injurious (containing no sharp pieces that could wound the animal), dust free and free from living organisms (especially those that cause disease, which implies that it must be sterilisable).

— Absorbent, so that it soaks up urine and spilt water, covers faeces to prevent the animals from becoming soiled and slows the release of ammonia[4].

— A thermal insulator, to reduce conduction of body heat through the floor of the cage.

— Comfortable for the occupants.

— Readily available, so that sufficient quantities are always available.

— Packed conveniently for handling and storage.

— Easily disposable: the method of disposal depends on what the animals have been used for. If they are stock animals, the soil can be composted; if the animals have been exposed to any chemical, infectious or other contaminant, the bedding must be incinerated.

In addition to the list above, bedding materials should be inedible, should not stain and should be easy to remove from cages when cleaning out.

Nesting materials share many of the same properties as bedding materials, with the obvious addition of enabling nest building.

6.3.2. Examples of bedding and nesting materials

A wide variety of bedding and nesting materials are available from laboratory animal suppliers. Users must select those that are most suitable for their own circumstances.

All bedding and nesting materials, except Vetbed™, are of plant origin and are therefore biodegradable. They are wood products, paper products, cellulose or dried plants.

Bedding that is placed on the floor of a cage is referred to as contact bedding. If it is used in a tray that goes under a perforated floor, it is referred to as non-contact bedding.

Wood products

These are wood chips and shavings of various grades.

Wood for bedding should come only from soft, white wood (e.g. aspen species), because some hardwoods contain toxic chemicals that could harm the animals. Specialist suppliers ensure that the wood has not been chemically treated with preservatives and have analyses carried out to determine the levels of other possibly harmful contaminants. Specialist suppliers will also grade products to the desired particle size and exclude dust and large, potentially harmful, pieces.

Wood materials are dried to reduce the moisture content to less than 10%, so they will absorb considerable amounts of moisture before they become soggy. The degree of absorbency is related to the particle size; shavings are less absorbent than smaller particles (**Figure 6.10** and **Figure 6.11**). Wood is a good insulator and, in particle form, the air trapped between the particles increases its insulation properties.

[4] Ammonia is produced by bacteria working on urine and faeces. These nitrifying bacteria work best in damp conditions and are inhibited in dry conditions. Very absorbent bedding slows the production of ammonia, which lowers the concentration in the cage and room. Ammonia is an irritant to the respiratory system

Figure 6.10. Wood chips

Source: Datesand

Figure 6.11. Wood shavings

Source: LBS Biotech

A continuous supply of wood products of the desired quality and in the desired packaging (e.g. compressed, shrink-wrapped or steam-permeable bags) is available. Wood products can be used as contact or non-contact bedding. The cost of the material depends on the quality of the product, the extent of the chemical analysis and the packaging that is required.

Most species can use softwood wool to construct nests, and shavings can be used by species like rats that construct open nests.

Paper and cellulose products

The manufacturing process of these products ensures that the paper and related cellulose fibre materials have a very low microbial load. They are easily stored, can be sterilised if necessary and can be disposed of by incineration. A major advantage of using these products is the very low levels of dust, and the consequent reduction of allergens. The material is specifically manufactured so that its quality is constant.

Although clean waste paper (e.g. shredded office paper) may have limited use with some larger farm animals, it is not recommended in laboratory animal facilities. This paper may be too harsh, leading to sharp edges that can cause injuries to animals, and the inks may be toxic. Some paper products available from specialist suppliers are recycled, but these are treated to ensure that they are harmless. Paper sheet can be used as a non-contact bedding and is useful when it is necessary to estimate food wastage, find vaginal plugs, or investigate urine and faeces production (**Figure 6.12**).

Figure 6.12. Paper sheet used as a non-contact bedding

Source: Stephen Barnett

Other specifically manufactured paper and cellulose bedding materials in chips, strand or compressed form are available. They are cut and folded to provide a soft comfortable substrate with good insulating properties and very good absorbency. They can be used as contact bedding and nesting materials (**Figure 6.13**). Paper wool and shavings make good nesting materials (**Figure 6.14**).

Figure 6.13. Cellulose-based bedding material

Source: LBS Biotech

Figure 6.14. Paper shavings

Source: LBS Biotech

Corncob

Corncob is produced by crushing the maize cob after the maize has been extracted; the resultant particles are graded into various sizes (**Figure 6.15**). The bedding is a good insulator and appears to be comfortable for the animals. Urine drains through the bedding to the bottom of the cage and is absorbed by the bottom layer of corncob, keeping the animals on the top clean and dry. The absorbency of corncob is very good, and animals need cleaning out less often than those bedded on wood products. It also has low dust levels. However, even the finest corncob may be too harsh for neonatal rodents.

Figure 6.15. Corncob

Source: LBS Biotech

Other nesting materials

Straw

Straw is traditionally used for farm animals. It is not absorbent, but it does allow the urine to drain through, so the top stays dry. Even the softest straw is too harsh for small laboratory species.

Hay

Hay is a natural nesting material and food for many species. Good-quality meadow hay should be free from weeds and will provide a soft nesting material for animals; however, all hay contains microbiological contaminants and should be sterilised, preferably by irradiation, before being given to laboratory animals. Rabbits will build a nest with it and it gives privacy to guinea pigs. Both species will eat it, so it needs to be replenished frequently (**Figure 6.16**).

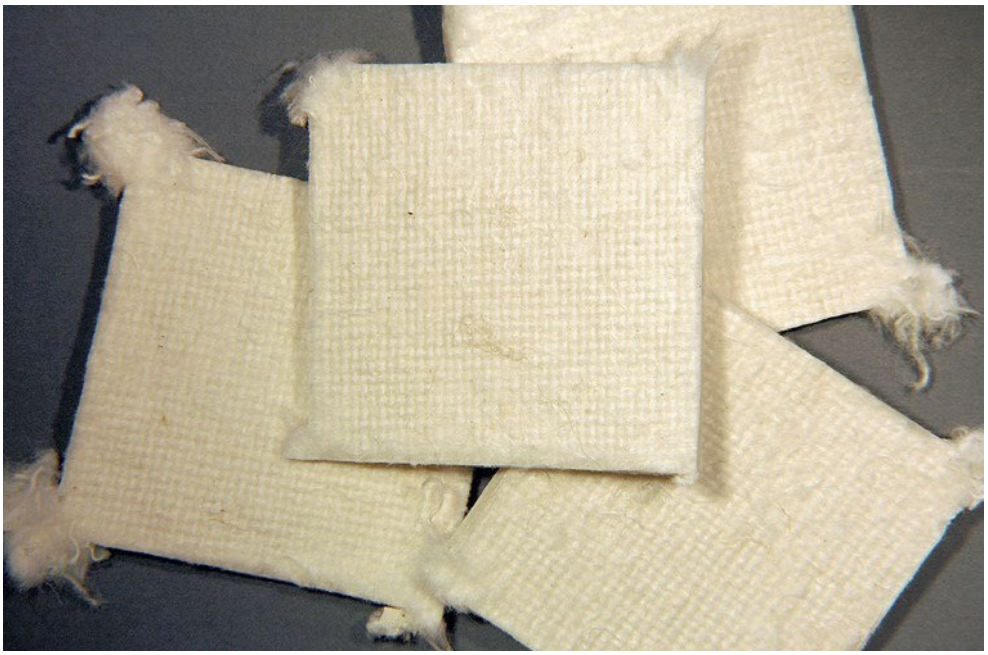
Figure 6.16. Young rabbit in a nest made with hay and lined with the doe's fur

Source: Stephen Barnett

Cotton fibre

Cotton fibre nesting material is supplied in 5 × 5 cm pieces, which can be added to the cage so the animal can shred them and construct nests, although some mouse strains seem unable to use them. The material is soft, non-irritating, inert and non-digestible (**Figure 6.17**).

Figure 6.17. Cotton fibre nesting material, which rodents are able to shred to make nests



Source: LBS Biotech

Vetbed™

Vetbed is a manufactured material made of a soft fibrous material backed by latex. Liquid drains through, leaving the surface dry. It can be machine-washed many times. It was originally used for dogs and cats, but it is suitable for any animal and in many circumstances, for example lining transport containers and providing a comfortable bedding for whelping and when recovering from surgery (**Figure 6.18**).

Figure 6.18. Bitch with pups on Vetbed™

Source: Pfizer UK

6.4. Sterilisation and disinfection

6.4.1. Sterilisation and disinfection techniques

Sterilisation and disinfection techniques are used alongside general cleaning to achieve a higher level of hygiene. Although all methods of cleaning will remove some of the causes of disease, sterilisation is designed to kill, destroy or remove all disease-causing agents, and disinfection is designed to reduce their number to a level where they are unlikely to be able to cause disease.

Sterilisation and disinfection reach maximum efficiency if they are applied to clean surfaces, so they are normally used after materials have been thoroughly washed. However, there are occasions when autoclaves are used to treat dirty materials, for instance when soiled bedding leaves an isolation or quarantine unit housing infected animals. The bedding is placed in sealed containers inside the unit and passes out of the unit through an autoclave, rendering the outer container safe to transport to an incinerator. The number of pathogens inside the container will also be greatly reduced in number.

Disinfectants may also be used in dirty conditions, for example in footbaths, where the aim is to reduce the risk of introducing pathogens into or out of the facility/room/pen on the foot-ware of people. It is important to select a disinfectant that is tolerant of organic material in these circumstances.

6.4.2. Definitions used in connection with sterilisation and disinfection

— **Sterilisation** is a process that results in the complete destruction or removal of all living organisms. The sterilisation process must be validated, which means tests must be carried out during and/or after the process to ensure sterility has been achieved (see **Chapter 6.4.3** monitoring autoclaves).

— **Disinfection** is a process that removes the causes of infectious disease. Disinfection does not destroy all forms of pathogenic organisms; it cannot be relied on to kill bacterial spores. The intention is to reduce the numbers of organisms to levels where there are too few to cause disease.

— **Pasteurisation** is a form of disinfection that kills vegetative forms of bacteria (i.e. those that are growing, not spores). This is done by rapidly raising the temperature to 70–80°C, followed by rapid cooling. The process is used to treat food because the times and temperatures used are effective in reducing the number of microorganisms, but do not damage nutrient quality. Pasteurisation was originally introduced to kill tubercle bacilli (the organism that causes tuberculosis) in milk. The pelleting process for laboratory animal diets achieves pasteurisation, reducing the number of viable microorganisms in the diet from 5×10^6 per gram in raw ingredients to as few as 5×10^3 per gram in expanded pellets.

— **Fumigation** is the use of gases or vapours in order to achieve disinfection/sterilisation. It is used to treat rooms and other spaces to clear them of potentially harmful organisms. Formaldehyde and hydrogen per-oxide (VHP) are commonly used fumigation agents; both are dangerous and must be used only by trained personnel in accordance with an established protocol. Hydrogen peroxide vapour is produced in a VHP generator (**Figure 6.19**). In addition to its use in decontaminating rooms, it is used in pass locks, where goods are passed through a barrier. Computers and other electronic equipment can be left in situ while decontamination with VHP is being carried out (Krause *et al.*, 2001).

Figure 6.19. Loaded VHP decontamination unit

Source: Tecniplast UK

— **Fogging** is the process whereby a fine mist of disinfectant is sprayed around an area to decontaminate it. The mist is produced by forcing disinfectant solution through the nozzles of a fogging machine (**Figure 6.20**). The nozzle rotates so that the whole area is covered. It can be used in rooms only when electronic equipment has been removed.

Figure 6.20. Fogger

Source: Stephen Barnett

6.4.3. Sterilisation

Sterilisation can be achieved by the use of dry heat, wet heat, chemicals, filtration and radiation.

Dry heat

To ensure that dry heat achieves sterility, materials must be exposed to high temperatures for a constant defined period. Dry heat has until recently had limited use in laboratory animal facilities because the material being treated is often damaged by the high temperatures needed. However, modern technologies including precise heat level control systems have expanded their use in animal facilities. The following are examples of the methods used to apply dry heat.

— **Specialist dry heat sterilisers**: are capable of sterilizing animal facility equipment including cages and racks. Sizes vary but some are capable of treating multiple cage racks (**Figure 6.21**). Advantages over traditional autoclaves include lower power consumption, no requirement for steam generation or vacuum, so all that is needed an electrical supply. Installation is generally much easier than an autoclave as the machine is assembled in a modular form with no need for a floor pit or large heavy steam jacket. Treatment cycle times vary depending on the equipment to be sterilised but a typical example for IVC racks is 90 minutes at 140°C.

Figure 6.21. Dry heat steriliser

Source: MMM Medical Equipment UK

— **Flaming**: in this method the material to be treated is exposed to a naked flame, from either a Bunsen burner or a flame gun; a temperature of 500°C is reached with these techniques. There are obvious dangers with flaming. It has some use in microbiology laboratories and has been used in outside dog pens to eliminate endoparasite eggs, but has little value in laboratory animal facilities.

— **Hot-air ovens**: metal or glass can be sterilised by exposure to temperatures of 160–180°C for one to two hours in a hot-air cabinet. The whole process takes much longer than one or two hours because the cabinet will take time to reach the correct temperature and then must be allowed to cool down afterwards before material can be removed. In hot-air ovens, the items being sterilised must be wrapped in an impervious, non-flammable material, such as aluminium foil, so they remain sterile until they are used.

— **Incinerators**: incineration is a dry-heat method used to render waste material from animal facilities sterile. Incinerators are fuelled by electricity, gas or oil and are built to reach temperatures in the region of 1000°C. At this temperature, a wide range of materials, including carcasses, bedding and sharps, are reduced to an inert ash. Even highly infective materials are rendered sterile, although care must be taken when transporting infective materials to the incinerator. Incinerators are expensive to install and their installation and use are strictly regulated because of the threat they pose to the environment.

Wet heat

Wet heat (also referred to as moist heat) for sterilisation involves the use of steam. Steam is produced when water is heated to its boiling point; the temperature at which water boils depends on the air pressure it is exposed to. At atmospheric pressure, water boils at 100°C (atmospheric pressure arises from the mass of air pressing on all objects on earth; at sea level it is 101.325 kPa). If the pressure is increased, water boils at a higher temperature (**Table 6.2**).

Steam produced at atmospheric pressure is an efficient disinfectant, but cannot be relied on to achieve sterilisation; however, if it is boiled at a higher temperature, for instance at 121°C or 134°C, even bacterial spores will be destroyed.

Latent heat is the property that makes high-pressure steam an efficient sterilising medium. Latent heat means hidden heat; it is heat energy that does not register on a thermometer, but is used to keep the water molecules in the air. The higher the boiling temperature, the more latent heat there will be. It is not the higher temperature that destroys the infectious agents, but the release of the latent energy in steam as it condenses on the objects that are exposed to it. An autoclave is used to maintain steam under pressure.

Table 6.2. Steam sterilisation: relationship between temperature, pressure and time

Temperature (°C)	Pressure (kPa)[5]	Holding time to achieve sterilisation (minutes)
120	101	Not applicable
121	103	15
126	138	10
134	207	3

Source: Stephen Barnett

Autoclaves

Autoclaves are pressure vessels in which objects can be exposed to steam produced above atmospheric pressure (**Figure 6.22**). To work efficiently, steam must come in contact with the infectious agent. This means that all air must be removed from the chamber and the objects being

[5] Pa, pascals (the SI unit of pressure)

treated must be physically clean (e.g. cages are sterilised after being through a cage wash, unless the circumstances described in **Chapter 6.4.1** apply. Small materials that are not to be used immediately after sterilisation must be wrapped in autoclave bags so they remain sterile after the autoclave is open. These bags are permeable to steam when they are heated, but become impermeable to microorganisms when they cool.

Autoclaves normally have a door at each end of the chamber: one for loading the non-sterile items (called the dirty side), and another that opens into a clean room for sterile goods (the clean side). Once the chamber has been loaded and the door closed, the sterilisation cycle can start (**Table 6.3**); the air is removed and steam is introduced. This is done in a series of pulses: some air is sucked out and some steam introduced, then more air is pumped out and more steam introduced until all the air is out and the chamber is full of steam. The vacuum ensures even penetration of steam throughout the chamber, including between the particles of porous materials, such as bedding. At the end of the sterilisation cycle, another vacuum is pulled that removes the steam and has the effect of drying the load.

Figure 6.22. Large autoclave

Source: Sychem

Table 6.3. Stages in the autoclave cycle

Stage	Description
Pulling the vacuum	Sucking air out and introducing steam in pulses
Increasing pressure	Building up pressure until it reaches the desired level. This depends on the type of cycle being run
Sterilisation stage (holding time)	The time for which material must be exposed to the steam before sterilisation is achieved
Ventilating steam	Pulling a vacuum to remove steam
Drying	Returning to atmospheric pressure and drying the load

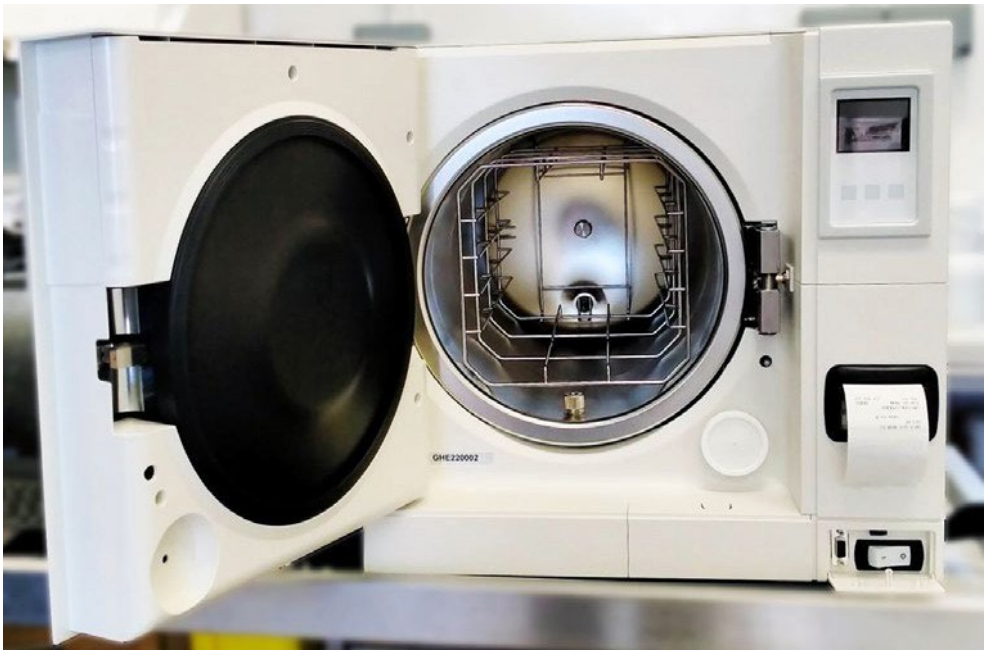
Source: Stephen Barnett

Autoclaves are electronically controlled in order to make them more efficient and to prevent accidents due to human error. Electronic sensors or thermocouples enable conditions to be monitored

throughout the cycle. This information can be viewed on monitors and/or printouts on the front of the autoclave. Different autoclave cycles are available on modern autoclaves, so the operator can choose the appropriate one for the material or load they are processing.

Smaller autoclaves (**Figure 6.23**), suitable for light loads such as surgical instruments, are available. Some are of the downward or upward displacement type, which means that, instead of being fitted with a vacuum to extract air, steam is introduced at the top or bottom of the chamber, and it pushes the air out of an exhaust valve at the opposite end. When a heat sensor in the exhaust registers the temperature of the steam, the valve is closed. It is assumed that all the air has been pushed out and the chamber is full of steam These autoclaves are not suitable for porous loads, for example food or bedding.

Figure 6.23. Small benchtop autoclave



Source: Sychem Ltd

Autoclaves use a combination of high temperatures and pressures, which means they are potentially dangerous pieces of equipment. It is essential, therefore, that they are operated in accordance with manufacturers' instructions and are regularly serviced.

Monitoring autoclaves

Although autoclaves are fitted with internal monitoring devices, other methods of monitoring are often used to confirm efficient operation. They are either chemical or biological indicators. They are placed around a load being sterilised and are read when the load is opened. Several types of monitors are available; three are mentioned here.

1. Autoclave tape (**Figure 6.24**). This is masking tape impregnated with strips of a chemical that changes from clear to black/brown when exposed to steam. This tape can be used to seal autoclave bags. The result is easy to read, but it is only an indicator that the items have been exposed to steam. It does not guarantee that they have been exposed to the required temperature, or for the required amount of time.

Figure 6.24. Autoclave indicator tape.
Top: appearance before autoclaving.
Bottom: appearance after autoclaving

Source: Stephen Barnett

2. Autoclave indicator strips (**Figure 6.25**) illustrates one of the several types of strip available. These can be placed in different locations around the load to ensure that the steam reaches everywhere. They are more accurate than autoclave tape because they change colour only when exposed for the required amount of time. Different strips are available for different autoclave cycles.

Figure 6.25. Indicator strip. The yellow dot changes to blue after the temperature has been reached for the required amount of time

Source: Stephen Barnett

3. Spore strips. These are strips of paper impregnated with bacterial spores from a species that do not cause human or animal disease (*Geobacillus stearothermophilus*). This species of bacteria can tolerate high temperatures. The spore strips are arranged around the load and, when the cycle has ended, they are sent, with a control strip that has not been in the autoclave, to a microbiological laboratory where they are cultured (i.e. placed in conditions where they should grow). If the autoclave cycle has been successful, the control strip should grow, but the one that has been in the autoclave should not. The culture takes a few days, so the test is most useful if the sterilised items are packed in autoclave bags and are not going to be used immediately. It is also a useful periodic test for any autoclave to check that it is working well.

Versions of these monitors are available for different autoclave temperatures and for different forms of sterilisation, for example, dry-heat cabinets, VHP, irradiation.

Chemical sterilants

Very few chemicals can be relied on to achieve sterilisation. The few that can are very toxic and should be used only in controlled conditions by trained staff. They usually need long exposure times to kill bacterial spores and can damage metals and plastics. Peracetic acid is an example of a chemical sterilant. It is used as a surface sterilant for isolators and their supplies.

Filtration

High efficiency particulate air (HEPA) filters are commonly used in Heating, ventilation and air conditioning units (HVAC) and in IVC units. These filters can remove up to 99.97% of airborne particles. Liquids can also be sterilised by filtration (see **Chapter 7.8**).

Radiation

Ionising radiation, emitted in the form of gamma rays (from a cobalt-60 source), is a very effective sterilising agent. Gamma radiation is extremely dangerous and, at the strength used to sterilise materials, would be lethal to humans and animals; exposure has to be carried out in specialist legally licensed units. Many materials used in animal units are treated with gamma radiation, for example food, bedding, hypodermic syringes and needles.

UV radiation will also achieve sterilisation, but, as UV rays have very poor penetration ability, they can be used only for surfaces or very thin layers of material. UV rays can also be used to sterilise the air in airlocks, cage-cleaning stations and safety cabinets. It is essential that the UV source is switched off when people are working in the area.

6.4.4. Disinfection

A regular programme of disinfection is essential to prevent infectious agents from entering and spreading within animal facilities. The aim of disinfection is to reduce the number of infectious agents in the environment; it cannot be relied on to achieve sterilisation.

Disinfection can be achieved by the application of heat and by chemical means.

6.4.5. Heat

Heat methods involve the use of water, either boiling or as steam produced at atmospheric pressure. Immersion in boiling water for 30 minutes has been used to treat surgical instruments that are used for very minor surgical interventions. The instruments must be thoroughly cleaned before boiling. This treatment will not render the instruments sterile.

Atmospheric steam applied with a steam lance can be used to disinfect pens or large cages.

Steam chests can be used to disinfect caging and equipment. As the name suggests, these are cabinets into which steam, at atmospheric pressure, can be introduced. Exposure times of approximately one hour are necessary to achieve adequate disinfection.

6.4.6. Chemical disinfection

Routine treatment with chemical disinfectants is used in all laboratory animal facilities to ensure that any pathogenic agents that may be present are not allowed to increase to levels at which they can cause disease. Choosing the most suitable disinfectant to use in particular circumstances is fundamental to controlling infectious agents (Ingraham and Fleischer, 2003). The following are some of the factors that need to be taken into account when choosing a disinfectant.

— Range of activity.

  A major requirement of a disinfectant is that it destroys pathogenic agents. If it is for general use, it should have a wide range of activity, which means that all unwanted agents are susceptible to it. If the need is to control specific pathogens, a disinfectant that has a narrower range of activity, but is known to destroy the pathogen(s) of concern, will be more appropriate.

— Tolerance of dirt and organic matter.

  In order to work most effectively, the disinfectant must come in contact with the pathogen. Organic matter (e.g. fats, oils, body fluids) can act as a barrier between pathogens and disinfectants; organic matter can also chemically combine with the active components in the disinfectant, leaving less to combat the pathogens. To prevent this, the object or area being treated should be thoroughly cleaned before disinfecting. Some disinfectants are tolerant of organic matter; these should be used where dirty situations are unavoidable, for example in footbaths.

— Resistance to disinfectants.

  Resistance arises when a genetic mutation occurs in a pathogen that blocks the disinfectant's ability to destroy it. Once it is established, the resistance is passed to all the offspring of the pathogen and, in some cases, can be passed to other mature pathogens. Resistance can arise to most disinfectants, but some are better at combating it than others. The way in which disinfectants are used may reduce the likelihood of it happening. For example:

  - disinfectants must be made up fresh; when used, old disinfectant must not be topped up with fresh;
  - at the end of a session, unused disinfectant should be discarded safely and all the cleaning utensils should be cleaned, preferably with a different type of disinfectant method;
  - changing disinfectants periodically can ensure resistant strains do not persist.

— Other factors affecting the efficiency of disinfectants

Disinfectant can be inhibited or enhanced by a number of other factors, for example:

- mixing certain types of disinfectants and detergents together – acid or alkaline conditions; o temperature range – many are enhanced by higher temperatures, but a few are inhibited;
- exposure time – some microorganisms are killed very quickly whereas others need to be exposed for much longer.

Safety

Chemical disinfectants are designed to destroy living organisms, so must be used carefully. Appropriate PPE should be worn when working with them, particularly when diluting concentrated stock solution to use dilution (that is the concentration that is required for use in the unit).

Disinfectants, other than those designed to treat the skin (antiseptics), should not come in contact with animals; not only will their health be compromised, but experimental results could be affected. Equipment that will come into contact with animals must be thoroughly rinsed after being disinfected.

Manufacturers' data sheets will detail all the relevant information, including range of activity, hazards, safe use and disposal of their products. All disinfectants must be used in accordance with local rules and SOPs.

6.4.7. Properties and uses of disinfectant groups

An outline of the properties and uses of some of the major groups of disinfectant is given below, but be aware that, within most groups, there may be several disinfectants, each with a different range of activity.

Disinfectants are grouped according to their major chemical component or mode of killing. The following terms are used in relation to disinfectants:

— cidal (e.g. bactericidal) means the agent is killed;

— static (e.g. bacteriostatic) means the agent is prevented from replicating.

Aldehydes

Example. Formaldehyde.

Properties. This agent is bactericidal, bacteriostatic, sporicidal, fungicidal and virucidal, being more active in hot solution. Use dilution can vary from 1% to 40%, depending on method of use, but it breaks down and becomes inactive quickly once diluted. It is highly toxic and should be used for fumigation only in controlled conditions.

Uses. Fumigation (when the formaldehyde is used as a 40% solution in water) (see **Section 6.4.1**).

Halogens

Halogens include disinfectants whose active ingredient is either chlorine or iodine.

Examples. Sodium hypochlorite and iodophors.

Sodium hypochlorite

Properties. The efficiency of sodium hypochlorite is measured in parts per million (ppm) of available chlorine. It will kill vegetative bacteria, fungi and viruses at low concentrations (up to 500 ppm); at this concentration it has low toxicity. It will rapidly kill bacteria spores at very high concentrations (2 500 ppm), but this concentration is both corrosive and an irritant to mucous membranes and skin, so should be used only in controlled circumstances. Activity is reduced by a factor of 10 for every unit of pH above 7. It is inactivated by light and some metals.

Uses. It can be used to treat water and feeding and watering utensils. It can also be used for animal cages and ancillary equipment when combined with a compatible detergent. Chlorinated detergents can be used in cage-washing machines.

Iodophors

Properties. Iodophors are effective for a wide range of bacteria, fungi and viruses. They will kill spores, but need a long contact time. Activity is enhanced by an acid pH and by heat; they have low toxicity. At high concentrations, iodophors have a good tolerance to organic matter.

Uses. Skin disinfection, footbaths and dunk tanks, and cleaning surfaces.

Oxidising agents

Examples. Hydrogen peroxide, Virkon™.

Properties. These disinfectants destroy pathological agents by oxidising their proteins and fats.

Hydrogen peroxide

This disinfectant will kill bacteria, fungi and, at high concentrations, spores. It destroys some types of virus (non-envelope viruses), but its effect on envelope viruses is variable, as is its effect on mycobacteria.

Uses Hydrogen peroxide can be used in liquid form or as a vapour. In vapour form it is used as an alternative to formalin for fumigating rooms, airlocks and safety cabinets.

Virkon

Virkon is reported to be active against bacteria, including spores, viruses and fungi. It can be an irritant and is corrosive to metals if exposed for long periods.

Uses. It can be used in a 1% solution to disinfect solid surfaces and is used as part of the decontamination process in safety cabinets.

Phenol and its derivatives

Properties. These agents are bactericidal and fungicidal, and destroy some types of virus (envelope viruses), but have no effect on bacterial spores. Their activity is reduced by the presence of organic material and oils. They are more active in an acid pH and also when in hot solution. They are stable at use dilution, which is usually 1–2%, and have fair rinsing properties (meaning they will rinse away

easily). Phenolic compounds are toxic and corrosive, and have an unpleasant smell. They should not be used in areas housing cats and dogs, as these animals are particularly sensitive to them.

Uses. Treatment of surfaces (e.g. walls and floors) following the removal of gross soiling.

Quaternary ammonium compounds

Properties. Quaternary ammonium compounds (QACs) are selectively bactericidal and bacteriostatic. Some compounds are also fungicidal and virucidal. They are good detergents, but, because they are cationic detergents (molecules with a positive charge), their activity is reduced if they are mixed with anionic (molecules with a negative charge) or non-ionic detergents. Their activity is also diminished by hard water, rubber, plastics, oil and some metals. Activity is enhanced by an alkaline pH and heat. They are stable at use dilution, which is usually 1–2%. They have low toxicity and poor rinsing properties due to surface absorption. Numerous QACs are commercially available for use as disinfectants; the level of activity will vary considerably between the different types.

Uses. Treating surfaces (e.g. walls, floors and benchtops) following the removal of gross soiling; treating feeding and watering utensils (followed by thorough rinsing).

References

Ingraham, A. and Fleischer, T. M. (2003), 'Disinfectants in laboratory animal science: What are they and who says they work?', *LabAnimal*, Vol. 32, No 1, pp. 36–40.

Krause, J., McDonnell, G. and Riedesel, H. (2001), 'Biodecontamination of animal rooms and heat-sensitive equipment with vaporized hydrogen peroxide', Contemporary Topics in Laboratory Animal Science, Vol. 40, No 6, pp. 18–21.

Meller, A., Kasanen, I., Ruksenas, O., Apanaviciene, N., Baturaite, Z., *et al*. (2011), 'Refining cage cleaning routines: Comparison of cardiovascular responses to three different ways of cage change in rats', Laboratory Animals, Vol. 45, No 3, pp. 167–173.

Mongillo, P., Scandurra, A., Kramer, R. S. S. and Marinelli, L. (2017), 'Recognition of human faces by dogs (Canis familiaris) requires visibility of head contour', *Animal Cognition*, Vol. 20, pp. 881–890, https://doi.org/10.1007/s10071-017-1108-4.

Contribution of this chapter to the Three Rs

This chapter describes the processes of cleanliness, disinfection and sterilisation, which are all essential elements in protecting the health and welfare of laboratory animals. Selecting suitable bedding and nesting materials and understanding how to minimise the stress of cage changing contribute to the refinement of the process. The use of regular routines and SOPs ensures standardisation of husbandry procedures and that nothing is left out, further benefiting the animals.

Self-assessment questions

1. What contribution do SOPs make to the welfare of laboratory animals?
2. Describe three procedures that can lessen the stress of cage changing for laboratory animals.
3. How can the spread of infectious agents and allergens be controlled during cage cleaning?
4. Distinguish between sterilisation and disinfection.
5. How can the risk of the development of resistant strains of bacteria be minimised in an animal facility?

7. Nutrition

After reading this chapter, you should be able to describe:

— the nutritional requirements of the relevant animal species and explain how these can be met (learning outcome 3.1.5);

— how to provide water and an appropriate diet for laboratory animals, including the sourcing, storage and presentation of suitable foodstuffs and water (learning outcome 4.6).

7.1. Introduction

In the wild, animals have the freedom to fulfil their nutritional requirements by eating a variety of foodstuff that they forage. In laboratory animal facilities, they do not have that freedom, so they are completely dependent on animal care staff to provide them with a balanced diet, in the correct quantities and in the correct form. The food and water provided also have to comply with any experimental requirements.

Food supplied to laboratory animals must also be of low microbiological content, nutritionally consistent and free from contaminants (e.g. growth promoters or antibiotics) that may be added to the diets of other animals. To be sure that they meet the required standard, the diets must come from reliable, specialist suppliers who can provide evidence of the quality of their diets (e.g. by certifying each batch produced).

Providing food to laboratory animals gives the opportunity for care staff to interact with them and to note their physical conditions, ensuring that they are healthy and are eating well. When experimental demands allow, food can be used to enrich the environment, for example providing some dietary ingredients in the form of forage (e.g. wheat grains for rodents) or in forage boxes or food puzzles (see **Chapter 3**).

This chapter outlines the nutrients required to provide a balanced diet and covers the more practical aspects of feeding and providing water to laboratory animals.

7.2. Nutrients

The nutrients that must be present in the diets of animals are proteins, fats, energy sources (which can come from carbohydrates, proteins and fats), vitamins and minerals. Food also contains indigestible fibre that has no nutrient value but is a necessary aid for efficient digestion. Water is essential for animal survival; this will be considered later in the chapter.

7.2.1. Proteins

Proteins form the main structural components of cells and tissues, hormones, enzymes, antibodies and other biomolecules. They are constructed of small subunits called amino acids. Several hundred different amino acids have been found in nature, but only 20 are associated with proteins. The same amino acids are found in the proteins of all living organisms, but the proportions in which they are present differ.

Plants and bacteria can synthesise amino acids from inorganic chemicals (organisms that can do this are called autotrophs). Animals can convert some amino acids from one type into another (e.g. methionine can be converted into cysteine) and can build others, but there are 10 amino acids that

have to be included in the diet. These are called essential amino acids. **Table 7.1** lists the essential and non-essential amino acids used to construct proteins.

Other amino acids are found in animals' bodies, but they are not used to synthesise proteins; an example is taurine, which is required in the diet of a cat (see **Chapter 7.4**).

Table 7.1. Essential and non-essential amino acids used to construct proteins

Essential amino acids	Non-essential amino acids
Arginine[6]	Alanine
Histidine	Asparagine
Isoleucine	Aspartic acid
Leucine	Cysteine
Lysine	Glutamic acid
Methionine	Glutamine
Phenylalanine	Glycine
Threonine	Proline
Tryptophan	Serine
Valine	Tyrosine

Source: Stephen Barnett

Animals obtain their essential amino acids by digesting proteins from plant and animal sources, with some contributions from bacteria and fungi. The digestive process breaks down the links between the amino acids. Individual amino acids and short chains of a few amino acids (called peptides) are small enough to be absorbed through the epithelial cells that line the digestive system. They enter into the capillaries that lie beneath the epithelium and are transported through the circulatory system to tissues throughout the body, where they are reassembled to form new proteins that the animal needs.

7.2.2. Carbohydrates

Carbohydrates provide the main source of energy in the diet, but there are also contributions from protein and fat. Plants synthesise carbohydrate from carbon dioxide and water by the process of photosynthesis. There are many types of carbohydrates, ranging from simple, soluble sugars to complex molecules such as cellulose. Cellulose forms the cell walls of plants. Soluble carbohydrates are broken down by enzymes in the digestive system to form glucose, a small molecule that can pass through the epithelial lining of the digestive system and then into the circulatory system. From there it is transported to the cells in the body where energy is needed.

Animals do not have enzymes that can break down cellulose, but some bacteria do have them. Herbivorous animals, like rabbits and cattle, have developed a fermentation chamber in their digestive systems where these bacteria live in a mutualistic relationship with the herbivore. This enables herbivores to gain the maximum nutritional benefit from their plant-based diets. Their gut bacteria digest the cellulose and, as a by-product of their digestion, produce nutrients that the herbivore can utilise. In the case of rabbits, the fermentation chamber is the caecum, and the rabbit obtains the nutrients produced by the bacteria by eating special faecal pellets produced at night (a behaviour called coprophagy). Digesting the faecal pellets releases the nutrients produced by the bacteria, making them available to the rabbit.

In cattle and other ruminants (sheep, goats, deer, etc.), the fermentation chamber is the first compartment of the four-chambered stomach, called the rumen (**Figure 7.1** and **Table 7.2**). This contains a very large

[6] Arginine can be made in the body, but not in sufficient quantities to support normal growth rates.

population of bacteria that are able to digest cellulose. Some of the products of bacterial digestion are absorbed through the rumen wall. The animal also benefits from digesting the mass of bacteria that pass from the rumen with other food to be digested in the rest of the intestinal tract.

Figure 7.1. A cast of the four-chambered stomach of a cow, viewed from the right side. In life, the rumen fills most of the left side of the abdomen

Source: Stephen Barnett

Table 7.2. The parts of a cow's four-chambered stomach (from **Figure 7.1**)

Key	Organ	Function
A	Rumen	Described in the text
B	Reticulum	An extension of the rumen, with a similar function. The two together are often called the ruminoreticulum
C	Omasum	Contains many membranes arranged like the leaves of a book. Its main function is to absorb moisture and other nutrients from the digesta
D	Abomasum	This acts like the stomach of a non-ruminant animal
E	Duodenum	The start of the small intestine

Source: Stephen Barnett

7.2.3. Lipids/fats

Lipids are a group of organic compounds that are insoluble in water, but soluble in organic solvents. They include fats, oils, fatty acids and steroids. The basic unit of fat is made up of glycerol and from one to three fatty acids. These units bond together to make the different types of fat; their chemical and physical properties are determined by the number and types of fatty acids.

Most fatty acids can be synthesised in animal bodies from excess carbohydrates and proteins, but two cannot, and must be included in the diet. These essential fatty acids are alpha-linolenic and linoleic acids. Studies have shown that, when these are omitted from the diet of rats, they suffer scaly skin, poor growth and a decline in reproduction and lactation, and eventually die. All of the clinical signs are reversed when the essential fatty acids are fed to them.

Fats are necessary in the diet to provide essential fatty acids; they also act as a transporter of fat-soluble vitamins. There is twice as much energy in a gram of fat as there is in a gram of carbohydrate or protein.

7.2.4. Vitamins

Vitamins are referred to as micronutrients because they are required in very small amounts; nevertheless, they are essential to the health and well-being of animals. Vitamins are classified as being fat soluble (vitamins A, D, E and K) or water soluble (the B vitamins and vitamin C).

Fat-soluble vitamins can be stored in tissues, but water-soluble vitamins are quickly excreted, so the risk of suffering deficiencies if the vitamin is left out of the diet is greater. As long as animals are fed diets obtained from specialist laboratory animal diet suppliers, the risk of deficiency will be very small. **Table 7.3** lists some of the fat-soluble vitamins and their deficiency signs, and **Table 7.4** lists the water-soluble vitamins and their deficiency signs.

Table 7.3. Fat-soluble vitamins

Vitamin	Chemical name	Deficiency signs	Notes
A	Retinol	Impaired vision at night, loss of appetite, poor growth, decreased resistance to disease	Many animals can convert carotene to retinol
D	D2: ergocalciferol D3: cholecalciferol	Poor growth, swollen joints, curvature of long bones (e.g. femur, tibia) (this condition is called rickets)	Many animals can utilise both forms of vitamin D, but New World non-human primates must have vitamin D3
E	Tocopherol	Muscular degeneration, degeneration of testes, neurological and circulatory defects	
K	Quinone compounds	Failure of the blood to clot	Many intestinal bacteria can synthesise vitamin K

Source: Stephen Barnett adapted from Brian Lowe

Table 7.4. Water-soluble vitamins

Vitamin	Chemical name	Deficiency signs	Notes
B1	Thiamine	Loss of appetite, weight loss, general weakness	
B2	Riboflavin	Inflammation of the skin, mucous membranes and conjunctiva	
B6	Pyridoxine	Weight loss / poor weight gain, vomiting and diarrhoea, scaly skin	
B7	Nicotinic acid Niacin	Weakness, loss of appetite, slow growth, digestive disorders, bleeding in the large intestines, scaly coat	
B9	Folic acid	Anaemia, decrease in number of white blood cells, degeneration of epithelial cells	
B12	Cobalamin	Anaemia, nervous disorders, poor reproduction, poor growth	
C	Ascorbic acid	Loose teeth, bleeding gums, haemorrhages under the skin, stiff swollen joints, fragile bones	Only guinea pigs and non-human primates of the common laboratory species suffer deficiency signs; all the other laboratory animals can manufacture it in their tissues

Source: Stephen Barnett adapted from Brian Lowe

7.2.5. Minerals

Minerals are inorganic chemicals necessary for the normal function of the animal body. Some (e.g. calcium, phosphorus, sodium and chlorine) are required in relatively large amounts and are referred to as macro-minerals. Others (e.g. iron, copper, cobalt and iodine) are required in relatively small amounts and are called trace minerals.

Minerals are required for:

— Structure, e.g. calcium and phosphorus in the skeleton

— Ionic–osmotic balance e.g. sodium and chlorine have a role in conducting nerve impulses and water balance in the body.

Minerals are also constituents of biological molecules and enzyme systems; for example, iron is necessary for the synthesis of haemoglobin, iodine is required to produce the thyroid hormone thyroxine and cobalt is necessary for bacteria to form cobalamin (vitamin B12).

7.3. A balanced diet

A balanced diet is one that provides all of the nutrients an animal requires in a form and quantity that it can consume during its normal feeding period.

What constitutes a balanced diet will differ depending on species, physiological condition, age and experimental design. Examples are given below.

— Physiological condition: lactation is the most physiologically demanding activity that female mammals engage in. Pregnancy is also very demanding, and it is common for laboratory animals to be pregnant and lactating at the same time. This physiological demand must be supported by higher levels of nutrients. Growing animals and stud males also require higher levels of nutrients than stock animals.

— Age: as animals get older and less active, they have a tendency to increase their body weight. Studies in rodents, especially rats, have shown that restricting dietary intake avoids the increase in body weight, extends the lifespan and decreases the onset of age-related diseases (Goto *et al.*, 2007). Diets that are adjusted for older rodents, especially those on long-term studies, are available from laboratory animal diet suppliers.

— Species: herbivores have a higher demand for plant-based materials than carnivores. Some species have a requirement for a specific nutrient, for example non-human primates and guinea pigs for vitamin C, cats for the amino acid taurine and New World primates for vitamin D3 (see **Chapter 7.4.5**).

— Experimental requirements: some studies may require altered levels of specific nutrients, for example higher fat levels in studies on diet-induced obesity.

7.3.1. Formulation of diets

Nutritionists formulate diets to ensure that the nutritional requirements of laboratory animals are met through all stages of their life cycles. It is necessary to balance diets for the needs of the animals; in addition, certain ingredients must be balanced with each other, for example:

— protein/energy balance: animals eat to satisfy their energy needs (Coates, 1999), so if they have a diet that has all of the nutrients they require, but it is too rich in energy, they could stop eating before they have taken in enough of the other nutrients, leading to deficiency signs;

— calcium/phosphorus balance: if a diet contains enough calcium and phosphorus for an animal's needs but one of them is present in higher amounts, it will block the absorption of the other, leading to deficiencies.

Nutritionists formulate the diet to ensure that dietary ingredients are balanced.

It is not enough to feed laboratory animals a diet that provides for their needs and keeps them healthy; the diet must be formulated to ensure that there is minimal variability between each batch of the same diet. This means that exactly the same ingredients at the same levels are used to provide the same nutrients every time the diet is manufactured. Diets of this type are called fixed-formula diets and they are of importance for animals used in science because they ensure that any anomalies in experimental results cannot be attributed to the diet.

The formulation of diets manufactured to the precision required for animals used in science requires the expertise found only among specialist suppliers. They not only produce diets of the required nutritional standards, but also ensure that their facilities are free from growth promoters, antibiotics and other additives that could cause health issues or experimental inaccuracies.

7.3.2. Contribution of the dietary ingredients to the nutritional value

Cereals such as wheat, barley, oats and maize contribute carbohydrate, protein, some fats and vitamins to the diet. The amino acids available from cereal protein are the same as those found in animal tissues; however, animals require some of them at higher levels than cereals provide. Plants such as soya and linseed and animal products such as poultry meal, fishmeal and whey are added to supply the deficient amino acids. Fishmeal and soya are also added to make sure the required levels of essential fatty acids are achieved. Although natural raw ingredients contain all the nutrients the animals require, synthetic supplements of vitamins, minerals and specific amino acids are usually added to make sure that the required levels of the most variable nutrients are met, for example vitamin C for guinea pigs and non-human primates, and vitamin D3 for New World primates.

7.3.3. Diets

Most laboratory animal diet manufacturers supply the following types of diet.

— Maintenance diet, for weaned animals: this diet can be fed for most of their lives without the animal laying down excess fat.

— Breeding diets: these diets contain higher protein and energy levels to support the females, young and stud males in breeding colonies.

— Total-life diets: these are formulated with mid-range protein and fat levels that still meet the requirements of breeding and shorter-term maintenance. The advantage of this diet is that facilities only have to use one diet.

— Custom-made/experimental diets: these are formulated to meet specific study requirements, for example high-fat diets, vitamin-deficient diets or medicated diets. They are manufactured to order and usually supplied in small quantities (i.e. from 5 kg). They are usually made in a separate facility using equipment that can be thoroughly cleaned down after use to prevent contaminating standard diets.

Table 7.5 shows the average amounts of dry diet and water that should be offered to an adult male animal per day. The figures are given as a guide only; many factors affect how much an animal will eat or drink in a day. Some animals play with food and water, which is wasteful and can lead to hoppers and water bottles emptying. It is essential to keep a close watch on the body condition of each animal to ensure that it has an adequate food intake.

Table 7.5. Food and water consumption per adult male per day

Animal	Daily food consumption	Daily water consumption
Mouse	5 g	6 cm^3
Rat	15 g	35 cm^3
Syrian hamster	10 g	8 cm^3
Guinea pig	40 g	100 cm^3
Rabbit	150 g	500 cm^3
Ferret	65 g	45 cm^3
Cat (3 kg body weight)	200 g	500 cm^3
Dog (12 kg body weight)	400 g	1500 cm^3
Macaque (per kilogram of body weight)	50 g	75 cm^3

Source: P J Kelly, K G Millican and Pam Organ

7.4. Forms of diet

Food is presented in different physical forms to suit the requirements of the animal and experimental demands.

7.4.1. Pelleted diet

Diet is pelleted to reduce waste and to make it easier to handle. Two main types of pellet are produced: compression pellets and expanded pellets (see **Figure 7.2**).

Figure 7.2. Compression pellets (left) and expanded pellets (right)

Source: Stephen Barnett

Compression pellets are produced by mixing the raw ingredients and grinding them into a meal. Steam is added to make a mash, and the mixture is pushed through a pelleting press. The opposite end of the press is perforated with circular holes; this is called the die face. The mixture is pushed out of the holes (called dies), like pencils. Cutters cut the pellets free from the outside of the die. The maximum temperature the pellets reach in the pelleting process is 80°C. The die determines the shape and size of the pellet, and the cutter settings determine their length. The die is changed depending on the species to which the diet is to be fed; for example, rabbit and guinea pig diets have smaller pellet diameters (3 mm), as they feed by taking the diet into their mouths (ingesting) and grinding it with their teeth. Pellets fed to rodents other than guinea pigs are larger (10 mm), as they feed by gnawing their food.

Compression pellets are denser than expanded pellets (that means they get the same nutrient value in a smaller size); consequently, they take up less space in storage and in the food hopper. This means less 'topping-up' of diet pellets is required, which can be a significant advantage when animals are kept in IVCs or isolators.

Expanded pellets start the pelleting process in the same way as the compression pellets, but the mixture enters an expander instead of a pellet press. Prior to reaching the die face, the meal is pushed along a tapered barrel under high pressure where additional steam is injected. The mixture is then forced through a die face, as described previously. The combination of higher temperature and pressure means that, as the diet emerges from the die into the ambient temperature, it expands, forming a honeycomb-/biscuit-type pellet that is larger than the die hole.

Expanded pellets have the following advantages:

— the pellets are harder and do not crumble as easily as compression pellets, so there is less waste in bags and in cages;

— the higher temperatures (up to 150°C) reached in the pelleting process 'cook' the ingredients, producing a more homogeneous pellet and increasing its palatability;

— the extra heat reduces the microbial load to a greater degree than the compression pelleting process.

Rodent and rabbit incisors grow throughout their lives (known as permanent eruption) and they need to have hard material to gnaw on to stop them overgrowing. Both pellet types should be hard enough to keep the animals' teeth in good condition.

7.4.2. Powdered diet

Powdered diets are used when a study requires a test compound to be added to the diet. They enable the compound to be thoroughly mixed in so the animals cannot avoid eating it. The usual method of manufacture is to pass the animals' usual pelleted diet through a grinder. This ensures that the nutrient quality of the diet is the same as that of the normal food and the particle size is consistent.

7.4.3. Mash

Mash consists of pelleted or ground diet mixed with water. It is useful for feeding animals that are unable to cope with a normal diet, for example, following surgery or another procedural intervention. It is usually fed in low-sided containers on the cage floor.

The increased water content in the mash encourages rancidity and mould growth, so fresh mash must be made up immediately before feeding and must be changed at least daily. This, together with the fact that the mash containers must be washed daily, makes this a labour-intensive method of feeding.

Commercial diets in the form of doughs and gels are available, which may be a more convenient replacement for mashes, although these may introduce dietary variability within an experiment.

7.4.4. Additional processed diets

The raw ingredients used in the manufacture of laboratory animal pellets contain a relatively high level of microorganisms, but the pelleting process reduces the level of microorganisms so that the pellets are safe to feed to standard laboratory animals. However, some studies require animals to be housed within more stringent barriers (e.g. immunocompromised animals, SPF animals); consequently, the food they are fed must undergo further processing to lower the level of microbial contamination. The further processing is done by either irradiation or autoclaving.

— Irradiation: the diets are exposed to measured doses of gamma irradiation of between 10 kGy and 50 kGy.

— Autoclave: the diet can be autoclaved into a barrier unit, but the temperature of the process destroys the heat-sensitive nutrients of the diet (e.g. vitamins A, B1 and D), so normal diet must not be treated in this way. Autoclavable diets are available that have higher levels of the heat-sensitive vitamins so that, even though some are destroyed, enough are left to satisfy the animals' needs.

7.4.5. Fresh food

Fruit and vegetables

Non-human primates are often fed fresh fruit and vegetables to provide variation in their diets, and it undoubtedly provides environmental enrichment. If fresh food is fed at the same time as a pelleted diet, animals will eat the fresh food rather than the pellets, which could lead to nutritional imbalance. This problem can be overcome by feeding pellets in the morning and fresh food later in the day. It is not possible to feed barrier-maintained (e.g. SPF) animals with fresh food because of the risk of introducing infectious organisms into the facility. Manufactured food additives with the taste of fruit are available from specialist suppliers for use with barrier-maintained animals, where experiments allow.

Hay

Small herbivorous animals, such as rabbits and guinea pigs, benefit from the addition of hay to their diet. It provides some nutrients and aids digestion. Hay is available in several forms, for instance as baled hay or as hay blocks. Hay is a vector of disease-causing organisms that can be a threat to barrier-maintained animals, so before it enters the facility it should be irradiated or autoclaved.

Hay forms the major part of the diet of larger herbivores, such as sheep, goats and cattle. These animals are not usually housed within stringent barriers, so it is normal to feed unprocessed baled hay.

7.4.6. Special nutritional requirements

Some species have a need for one or more specific nutrients, and it is important to ensure that these are supplied in their diets. They will already be in diets obtained from laboratory animal diet manufacturers, as long as the diets have been stored correctly and used before the expiry. Examples of these special nutrients are given below.

Ascorbic acid (vitamin C)

Most common laboratory animal species can synthesise ascorbic acid in their tissues. Primates (including humans) and guinea pigs cannot, so it must be included in their diets. As it is a water-soluble vitamin, it is not stored in the body; therefore, it must be fed every day.

A deficiency of vitamin C (a disease commonly called scurvy) in the guinea pig will first show as a staring coat (ungroomed with hair standing on end) and a reluctance to move. In severe cases, there may be loose teeth, bleeding gums, haemorrhages under the skin and/or stiff and swollen joints. Long bones may break easily and death may follow if the deficiency is not rectified. In non-human primates, the deficiency signs are similar and include weakness, reluctance to move, bruising, weight loss and anaemia.

Vitamin D3 in New World primates

Vitamin D comes in two forms, D2 and D3. Most animals can utilise both vitamin D2 (ergocalciferol) and D3 (cholecalciferol), but New World primates can utilise D3 only. Vitamin D deficiency is mostly seen in young animals; the signs include poor growth, swollen joints and curvature of the long bones.

Taurine in cats

Taurine is an amino acid, but it is not found in proteins; it is called a non-protein amino acid. All animals require it, but can make it from other nutrients. Cats can make it, but not in enough quantities for their needs, so it has to be present in their diets. Deficiency signs are widespread, and include retinal degeneration, heart disease and reproductive failure.

7.5. Presentation of diet

The Latin term *ad libitum* (*ad lib*), which means 'at one's pleasure', is used to describe the practice whereby food and/or water is freely available to an animal at all times. The term 'restricted feeding' is used when food is available for part of the day only or when only measured amounts are fed.

In captivity, food is usually presented in containers; this keeps the food clean, minimises waste and is convenient. A variety of containers are used to accommodate the animals' feeding behaviours and the types of food they eat.

Bowls are used for animals that are likely to eat a whole ration immediately after it is presented (e.g. dogs), when mashes or seeds are fed and when animals are housed in pens. It may also be appropriate to present food in an open container on the floor to animals that have difficulty reaching a hopper, for instance sick animals or some genetically altered mouse strains. Hoppers are used for animals that eat little and often and when food is intended to last for several days (e.g. rabbits and rodents). The shape and size of the food container used will be influenced by the species, the pellet size, the age of the animal (and therefore the amount they consume) and the number of animals in an enclosure (**Figure 7.3, Figure 7.4** and **Figure 7.5**). Where more than one animal is housed in a cage, food should be presented over a wide area; animals are competitive feeders and some dominant

individuals will try to prevent others in the cage from feeding. The wider the area of food presentation, the less successful the dominants will be.

Figure 7.3. Mouse cage showing hopper

Source: Stephen Barnett

Figure 7.4. Rabbit hopper and water bottle

Source: Stephen Barnett

Figure 7.5. Food bowl on the floor

Source: Sarah Lane

Although it is usual to present diet in food hoppers and bowls, it provides environmental enrichment for some species if they have to work for their food; for example, fruit and pellets can be scattered over the floor of a non-human primate cage and they will be kept busy searching, or foraging, for it. Similarly, pellets can be put in forage boxes or hidden in toys.

7.6. Diet quality

Diet quality depends on the ingredients used, the manufacturing process, the packaging and the conditions the diet is kept in between manufacture and being eaten by the animal.

Specialist laboratory animal diet manufacturers analyse the raw materials that will be used in the diet to ensure that they meet the required nutritional and physical standards. Further quality checks are made during the manufacturing process. If the diet is being used for Good Laboratory Practice-regulated studies, more detailed analysis is carried out involving the levels of heavy metal contamination, pesticide residue and microbiological status, in fact anything that could affect the study results. The results of these analyses are available to the scientists and study directors.

The diet is packaged in sturdy bags that are able to withstand storage, transport and handling; if the diet is to be irradiated, the packaging used is able to exclude contamination post treatment.

Prior to use, diet should be kept in cool, dark, dry, well-ventilated conditions that are insect- and vermin-proofed in order to protect it from chemical deterioration, spoilage and deterioration by organisms. Provided that the food has been kept in accordance with the manufacturer's guidelines, the shelf life of pelleted diets should be up to nine months.

7.6.1. Chemical deterioration

Natural interactions between chemical components of foodstuffs and oxygen (oxidation) occur at a rate that depends largely on temperature. An ambient temperature of less than 15°C will reduce oxidation to an acceptable level. Vitamins and fats are particularly susceptible to this type of deterioration; fats become rancid, causing a reduction in palatability and deterioration in the nutritional value of the diet.

7.6.2. Contamination by organisms

Temperatures above 15°C increase the metabolic and proliferation rates of organisms that may spoil food. All such organisms require a source of water, and their activities are limited by low humidity. Dark conditions tend to discourage many flying insects. Details of some of the organisms that cause the deterioration of diet are given in **Chapter 7.7**.

If spoilt or contaminated food is fed to animals, there is a danger that it will no longer meet their nutritional requirements, and contamination from outside sources could give rise to poisoning or infectious disease.

7.6.3. Storage of diet

From the information above, it follows that food stores should be cool (less than 15°C), dry, dark, well ventilated, insect- and vermin-proof and easy to clean. To make sure that no contamination can take place, only food should be placed in the store, and dry diet (e.g. pellets) should be kept separate from fresh food.

Diet stores are designed so that walls are smooth, washable and without cracks or crevices where insects and other pests can hide. Hot water or steam pipes should not pass through the store, as they may raise the temperature. Rodent barriers (**Figure 4.7**), fly screens and electrical devices that attract flying insects with UV light, which causes them to pass through an electrified grid as they try to reach it, will help to eliminate pests (**Figure 4.6**).

Diet should be stored on pallets or duckboards off the floor and away from walls so that there is a good circulation of air around them, and to prevent condensation.

The following are some of the procedures that should be included in a food store routine:

— inspect food bags on delivery and reject any that are damp, damaged or discoloured or whose use-by date is near or has passed;

— do not allow opened food bags into the food store;

— record the delivery in accordance with local rules and stack the diet according to its use-by date;

— record temperature and humidity daily;

— handle the diet carefully and as little as possible, to avoid splitting bags and causing the pellets to crumble;

— clear up any spilt diet immediately;

— do not open bags in the main food store;

— carry out regular inspections of the store for signs of infestation;

— institute a regular cleaning programme.

The amount of diet issued to animal rooms should be limited because the warmer temperatures in animal rooms speed up deterioration. Storage containers in holding rooms should be completely emptied and cleaned before they are filled with the next batch, so that any pests that may be present in the dust at the bottom do not persist. Empty diet bags should be discarded, as they could attract pests.

7.7. Food pests

Food of any type is always at risk of being damaged by pests of many kinds. Food pests not only contaminate and consume food, but can also act as vectors of disease-causing agents. Following the recommendations mentioned in the previous section of this chapter helps to minimise the risk of infestation, but cannot eliminate it completely. It is important, therefore, to regularly inspect food wherever it is stored.

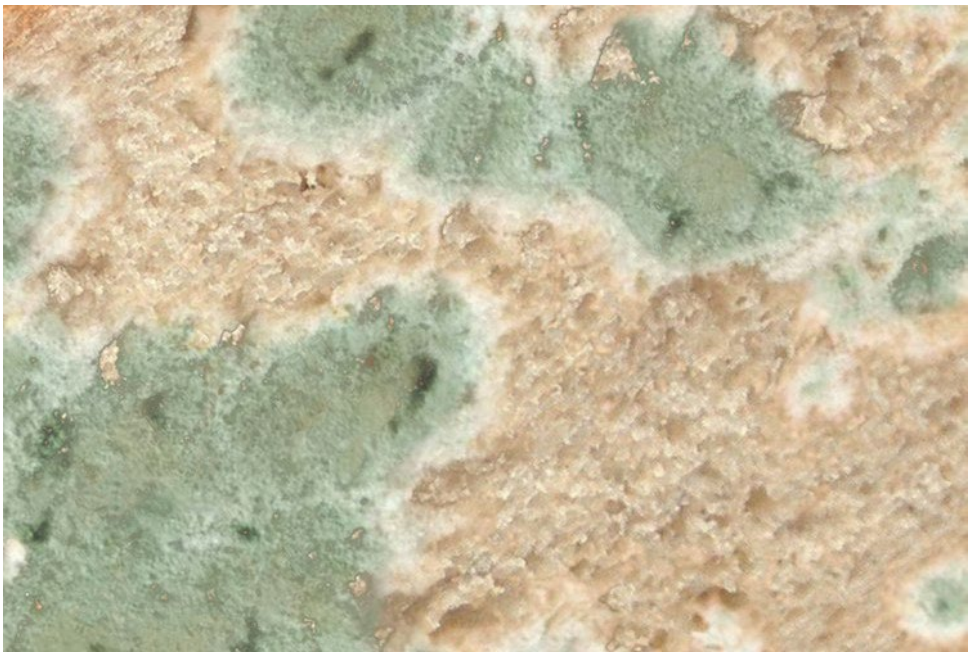
The most obvious sign of infestation is seeing the pests themselves, but they are often difficult to see: they may be too small or very good at hiding. Other general signs include smell, dusty diet, diet that weighs less than it should and holes in diet bags.

Below are a few examples of common food pests.

7.7.1. Moulds

Moulds are fungi and are therefore related to mushrooms. Like mushrooms, they consist of a mass of hairlike structures, called hyphae. The hyphae produce enzymes that penetrate the food to digest it, so they can absorb the nutrients. When the hyphae are sufficiently developed, they send up branches above the food (aerial hyphae), from the top of which new spores develop. It is only at this stage of development that it is possible to see the actual mould (**Figure 7.6**). However, its presence can be identified before this by the musty smell it produces.

Figure 7.6. Mould



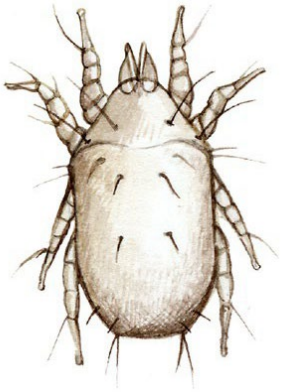
Source: Stephen Barnett

In addition to spoiling food, some moulds cause allergic respiratory disease in animals and humans if they are inhaled.

7.7.2. Flour mite

Flour mites are in the class of invertebrate animals called arachnids, so they are related to spiders. These mites are very small – females are 0.5 mm long and males are slightly smaller – which makes them difficult to see with the naked eye (**Figure 7.7**). En masse they appear as dust, but the dust appears to be moving. The activity of the mites damages the diet and contaminates it with their bodies and excreta. An offensive smell also indicates their presence. Mites need damp conditions to breed, but that need only be in a small area.

Figure 7.7. Flour mite



Source: IAT

7.7.3. Flour moth

Moths belong to the insect class of invertebrates, which also includes flies and beetles. The flour moth is 13 mm long with a wingspan of 20–25 mm (**Figure 7.8**). The adults settle on any surface during the night and start flying when it becomes light. They lay eggs near the diet, which hatch into caterpillars that are white with a brown head. The caterpillars trail a silken thread from a gland near the head. It is the caterpillars that cause damage to the food; in addition to consuming it, they contaminate it with their excreta and the silken thread.

Figure 7.8. Flour moth

Source: Shutterstock.com

7.7.4. Grain weevil

Weevils are similar to beetles, but the head ends in a slightly curved proboscis. The weevil is 3–5 mm long and dark brown to black in colour (**Figure 7.9**). The female lays up to 200 eggs at a rate of two or three a day. Each egg is placed in a small hole that the female has bored in the pellet or grain; once the egg is laid, the hole is plugged with food dust mixed with saliva. When they hatch, the larvae feed on the diet and then eat their way out, leaving a small hole (**Figure 7.10**).

Figure 7.9. Grain weevil

Source: Shutterstock.com

Figure 7.10. Damaged grain

Source: Stephen Barnett

Grain weevil damage can be extensive; they consume the food and cover it with excreta that renders it unpalatable. Their activity heats the diet, which speeds up both the deterioration of the diet and the development of the weevils.

7.7.5. Sawtoothed grain beetle

These insects get their name from the serrated edge at the sides of their thorax (**Figure 7.11**). They are 2.5–3.5 mm long, and dark brown in colour. The body is dorsoventrally flattened (which means

that they are flat from their back to the front). They lay their eggs on or near food; when the larvae hatch, they feed on damaged food. Their activity damages diet, reducing its quality and palatability. They, like most food pests, are vectors of animal-disease-causing agents.

Figure 7.11. Sawtoothed grain beetle

Source: Shutterstock.com

7.8. Water

Water makes up 50% to 70% of an animal's body and plays a major role in the physiological processes that enable living organisms to function. For example, water provides the medium in which most chemical reactions take place, it transports substances and cells around the body and it plays a role in hearing and sight. Water also has an important role in thermoregulation. A loss of just 15% of water from the body is likely to be fatal. Water is therefore an essential part of an animal's diet and should be constantly available to them.

Water quality is an important consideration when it is supplied to laboratory animals. Water supplied to humans has usually gone through public water treatment works that have rendered it safe for human consumption (this is called potable water), but it is not sterile. Although it may be adequate for some laboratory animals, it will not be good enough for all of them, particularly the small rodents kept under SPF conditions. Water can be further treated to render it sterile, for example by adding acid or chlorine, by passing it through a UV light source, by ultrafiltration using reverse osmosis systems, by autoclaving or by a combination of two or more of these.

7.8.1. Methods of presenting drinking water

The method of presenting water to animals varies depending on the drinking habits of the species and the volumes they consume. Experimental requirements and work routines can also affect the method used. Suitable methods of presenting water to various species are given in **Table 7.6**.

Open bowls

All animals can drink naturally from open bowls, although most are inclined to make a mess. Water in a bowl can be soiled or spilt, making the cage and the animals wet. Small animals may be at risk of drowning. Wherever possible, the bowl should be fixed to prevent overturning. Even so, it will need to be cleaned and refilled at least twice a day. For these reasons, bowls are not widely used; however,

they are the most suitable method for cats, and are sometimes used for dogs. Reducing the amount of open water in the cage limits wastage, contamination and mess.

Bottles

Bottles consist of a spout (or sipper tube) attached to an upturned bottle, which acts as a reservoir, ensuring that water is constantly available while limiting the opportunity for contamination and wastage.

Bottles provide an adequate water supply only if the orifice of the spout is of sufficient size to allow free flow when used by the animal, and is also small enough to prevent air from entering the tube when it is not being used. The mechanics are such that an animal cannot drink from a completely full bottle; conversely, if the air space is too large, the water may run out. This is because air expands at a much faster rate than water when it gets warm. A very small increase in ambient temperature (say 1°C) can cause enough air expansion to push the water out of the spout. The length of the spout is also an important consideration; it must reach low enough in the cage for the animal to reach it, but not so low that it comes in contact with bedding or nesting material, because this may cause it to empty.

The amount of water that should be provided to a cage or pen depends on the species, the number of animals and the size of the animals. Two smaller bottles can be better than one large one because, if one large bottle leaks, the animals are left with no water and a flooded cage; if one smaller bottle leaks, a supply of water is still available and the flooding is limited. Having two bottles ensures that a dominant animal cannot stop cage mates from drinking.

Water bottle systems are cheaper to purchase than automatic systems, but are more labour-intensive to maintain. Transporting water bottles can be heavy work, and removing the tops can lead to repetitive strain injury; however, there are automatic filling systems available to reduce these risks (see **Figure 6.9**).

Water bags

The water bag is a relatively new method of presenting water to smaller laboratory animals (**Figure 7.12**). It consists of a disposable plastic bag fitted with a reusable and sterilisable drinking valve, which replaces the bottle. The bags can be purchased already filled with sterile water, or a complete system can be obtained that allows the user to fill bags from their own water source (**Figure 7.13**). Using the bags eliminates the need for bottle-cleaning and -filling equipment and saves time, but does involve disposing of a large number of used plastic bags.

Figure 7.12. Water bag in a mouse cage

Source: Edstrom Industries

Figure 7.13. Apparatus for filling and sealing water bags

Source: Edstrom Industries

Automatic water systems

Automated water systems are widely used for laboratory animals. Treated water is distributed in pipes around the room; at various points there are outlets where a flexible tube from a rack can be connected so that the water can pass through the rack distribution system. At each cage, the distribution pipe ends in a drinking valve so that the animals can get access to the water (**Figure 7.14**). A range of valve sensitivities are available, from those suitable for small animals such as mice to larger and stronger ones suitable for pigs (**Figure 7.15**).

In order to prevent the water from stagnating in the distribution pipes, the system is regularly flushed. Flushing is usually controlled by a computer. The valves have to be checked daily to ensure that water is coming through to each animal. The whole system is cleaned when the rack goes through the cage wash.

Providing water through automatic systems saves staff time and effort and minimises the risk of injuries due to lifting bottles and removing their tops. If there is a need to monitor water consumption or add compounds to the drinking water of a single animal, bottles or bags have to be used.

Figure 7.14. Rat drinking from an automatic water nipple

Source: Edstrom Industries

Figure 7.15. Pig farrowing unit showing drinking nipples on the left of the opening. Note the two heights, one for the sow and one for the piglets

Source: Stephen Barnett

Table 7.6. Methods of presenting water

Animal	Open bowl	Water bottle	Automatic valve
Mouse		+	+
Rat		+	+
Hamster		+	+
Guinea pig	+	+	+
Rabbit		+	+
Ferret	+	+	+
Cat	+		
Dog	+		+

Note: cells with '+' mean 'method suitable for these species', while empty cells mean 'method not used for these species'
Source: Stephen Barnett

References

Coates, M. E. (1999), 'Nutrition and feeding', in: Poole, T. B. (ed.), The UFAW handbook on the care and management of laboratory animals, 7th edition, pp. 45-60. Blackwell Science, Oxford.

Goto, S., Takahashi, R., Radak, Z. and Sharma, R. (2007), 'Beneficial biochemical outcomes of late-onset dietary restriction in rodents', Annals of the New York Academy of Sciences, Vol. 1100, pp. 431–441.

Further reading

Lowe, B. L. (2007), 'Nutrition', in: Barnett, S. W. (ed.), Manual of Animal Technology, pp.175-197. Blackwell Publishing, Oxford.

Contribution of this chapter to the Three Rs

Ensuring that laboratory animals have a diet that provides for their nutritional needs is fundamental to their welfare. Monitoring food and water intake can provide an early indication of ill health in an animal. Food can be used as environmental enrichment. Preventing infestation of food protects animals from unpalatable food and possibly infectious agents.

Self-assessment questions

1. What are the characteristics of a fixed-formula diet?
2. Compare compression pellets with expanded pellets.
3. When might it be appropriate to feed animals with a powdered diet?
4. What measures should be introduced in a food store to keep pelleted diet in good condition?
5. List the advantages and disadvantages of presenting water to animals using:
    (a) water bags;
    (b) an automatic water system

8. Breeding common laboratory animals

After reading this chapter, you should be able to describe appropriate breeding programmes for common laboratory species (learning outcome 4.10).

8.1. Introduction

Most laboratory animal facilities buy in the animals they need from authorised breeders or suppliers, who are able to provide a range of species and strains of specific ages and of one sex, if needed. They will also supply documentation on the animals, detailing health and genetic status, and, as they have a large number of clients, they have a greater opportunity to find a user for the animals they produce, leading to less wastage. If animals have to be killed in authorised breeding establishments, tissues can be preserved for clients who need them for their research programmes.

There are, however, circumstances when it is necessary for user establishments to breed in-house, for example:

— to create genetically altered strains;

— to obtain rarely used species/strains or species/strains unavailable from commercial sources;

— for work involving fetuses or neonates;

— when there is a need to have direct control over the quality of the animals.

Breeding animals for scientific procedures is a complex matter. It involves understanding the physiology and behaviour of the species and the requirements of the scientists using them. It requires constant vigilance from animal care staff to ensure that no animal suffers if there is a problem during the breeding process, while being aware of the ethical imperative not to overbreed, as this results in surplus animals and a waste of life. It also requires great care in setting up breeding animals and keeping breeding records.

This chapter outlines the principles of setting up breeding colonies, including examples of mutant strains and the early development of the offspring.

Techniques specifically related to animals produced as a result of genetic modification techniques are covered in **Chapter 9 'Genetically altered animals'**.

8.1.1. Stocking densities

Throughout this chapter, mention is made of a suitable number of animals to put together in a harem, for example one male with three females, but stocking densities must also be taken into account. If the available enclosures are too small for the number of animals in the harem, a smaller harem must be set up (see **Chapter 5.2.1** and Annex III of Directive 2010/63/EU) (the Directive). The same is true for fish-breeding tanks.

8.2. Glossary of breeding terms

Familiarity with the language used in breeding makes the subject easier to understand, so this section explains the terms in common use. If there are any terms elsewhere in the text that are not clear, they should be explained in this glossary.

Table 8.1. Glossary of breeding terms

Age at first mating	The age at which animals are first paired for mating. This is usually later than the time at which the animals are first capable of becoming pregnant
Allele	See Heterozygous
Breeding season	The period in the year when animals that do not breed continuously come into breeding condition, so that they mate and become pregnant
Closed colony	A colony that does not take in any animals from outside; all new stock is bred from within. Closed colonies reduce the risk of disease being introduced into the colony
Colony	A group of animals of the same species kept together for a particular purpose (e.g. a breeding colony)
Culling	Removal of weak, old, sick or excess stock from a colony
Delayed implantation	If a female is under great physiological demand (e.g. if she is rearing a large litter), implantation of the growing embryo may not happen immediately. It can be delayed for about five days. This has the effect of extending the gestation period (see Implantation)
Economic breeding life	The time during which a breeding animal is producing offspring of the required quality and quantity
Embryo	Name given to developing young in the early stages of pregnancy (although embryologists use the term to refer to all stages of prenatal development)
Fertilisation	The union of the male and female gametes (sperm and egg) to form a zygote (see Zygote)
Fetus	Name given to developing young from the time they become recognisable
Fostering	Rearing of young by females other than their own mothers
Foundation Stock	An inbred strain is established after 20 consecutive generations of brother × sister matings. In order to produce enough offspring to use, at each generation after the 20th, offspring are removed and placed in an expansion colony. However, the original brother × sister mating system must continue, to ensure the genetic purity of the strain. This is the foundation stock.
Genetically altered animals (GAAs)	Genetically modified (transgenic, knock-out and other forms of genetic alteration) and naturally occurring or induced mutant animals
Genetically modified organisms (GMOs)	Biological organisms (animals, plants, bacteria, etc., apart from humans) whose genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination
Genetic monitoring	DNA analysis caried out on genetically defined strains to ensure that they have not become genetically contaminated
Genotype	The full complement of genes in an organism
Gestation period	The period between fertilisation and parturition
Harem breeding	A breeding strategy using one male and more than one adult female
Heterozygous/homozygous	Animals have two copies of each gene that codes for a particular characteristic, one derived from the male parent and one from the female parent. Each copy is called an allele. If the two alleles are identical, they are said to be homozygous, but if the alleles code for a different version of the characteristic, they are said to be heterozygous
Implantation	Embedding of the zygote into the lining of the uterus
Inbred strain	A strain that has been produced as a result of at least 20 consecutive generations of brother × sister mating or youngest parent × offspring mating. The offspring are as genetically similar as it is possible to be using breeding techniques They are useful for many types of research because the results of the experiments are not affected by genetic variation. Inbred strains are often weaker than non-inbred strains. They have smaller litters and are more susceptible to disease and environmental stress (a phenomenon called inbreeding depression).
Inbreeding	Mating of close relatives

Independent feeding	The point at which a young animal is capable of feeding itself
Knock-in strain	Animals that have DNA inserted in a specific location in their genome by genetic engineering techniques (a targeted insertion)
Knock-out strain	Animals that have a gene removed, replaced or disrupted from their genome by genetic engineering techniques
Lactation	Production of milk
Litter interval	The period between the birth of one litter and the birth of the next
Mating, copulation, coitus	Insertion of the penis into the vagina so that male gametes can be deposited in the appropriate place for them to fertilise the egg(s)
Oestrous cycle	The reproductive cycle in the female mammal. It is described as having four stages: pro-oestrus, oestrus, met-oestrus and dioestrus. The stages reflect the changes that occur in the female body so that she will mate, ovulate, and provide a site for the egg(s) to be fertilised and a place for the developing young to be nourished and grow. Note the difference in spelling of 'oestrous' (adjective) when describing the cycle and 'oestrus' (noun) when referring to the stage
Oestrus	The stage in the oestrous cycle when the female will allow herself to be mated (called 'heat' in some species). Mating occurs near to ovulation so that sperm is available to fertilise the egg when it is shed
Outbreeding	The deliberate avoidance of inbreeding. Selecting animals for breeding that are not related. This produces the widest possible genetic diversity within a colony
Ovulation	Release of eggs from the ovary. In some species, ovulation is spontaneous and occurs regularly after each oestrus. In other species it is induced, meaning that it is stimulated by mating
Parturition	The process of giving birth (in mammals).
Phenotype	The observable characteristics of an organism
Placenta	An organ derived partly from maternal tissue and partly from embryonic tissue that attaches the embryo to the uterus and through which nutrients and gases pass to the developing fetus and waste products are removed
Postpartum oestrus	The oestrus that occurs very soon after giving birth in some species. If males are with the females during parturition, they will mate during this oestrus, resulting in a shorter interval between the births of their litters
Pre-weaning loss	Number of young that die between birth and weaning
Pre-weaning mortality rate	The number of young dying between birth and weaning expressed as a percentage of the number born: $\frac{\text{Young died}}{\text{Young born}} \times 100$
Pseudopregnancy	Outward signs of pregnancy in a female when fertilisation has not taken place. The cause may be a sterile mating or it may occur when mating has not taken place. In most species it lasts for about one half to two thirds of the normal gestation period. In the jill (female ferret) and bitch (female dog), it is of the same duration as the gestation period
Random breeding	Selecting animals for mating without any regard to their relationship. To be strictly random, animals should be selected using random number tables or a random number generator. Some of the breeding animals may be related; others will not be. At each generation there will be a new distribution of related and unrelated mating
Species	In organisms that breed only asexually, a species is defined in terms of similarity of appearance, genetics and mode of life. The same is true of species that breed sexually, but they are also defined as a group that interbreeds to produce fertile offspring
Staging	A technique that is used to determine the stage of the oestrous cycle by visual observation of the vulva, especially in mice
Strain	A true-breeding subgroup of a species, whose members have a distinguishing characteristic that separates them from the rest of the species

Timed mating, arranged mating	Mating arranged so that young will be born on a known day, or to produce females that can be used to implant fetuses that have been genetically altered (i.e. mating may be observed or identified by the presence of copulatory plugs or when females are known to be in oestrus)
Transgenic animals	Animals that have had genetic material from an external source (exogenous) introduced into them by genetic engineering techniques so it joins with the existing DNA in a random manner
Vaginal smear	A small sample of cells shed from the wall of the vagina that can be used to identify which stage of the oestrous cycle the female is in. The smear is usually taken by introducing a very small amount of sterile saline in the vagina with a sterile pipette, sucking it out again, putting the liquid on a slide, drying it and studying it under a microscope
Weaning	Gradual change in diet of young animals from milk to solid food It is also the term used to describe the removal of young from their mother's cage when they are able to feed themselves
Zygote	A cell produced as a result of the union of the nuclei of the ovum and the sperm

Source: Stephen Barnett

8.3. Breeding systems

'Breeding system' is the term used to describe a set of procedures designed to produce animals of the desired type and number. It includes arrangements for mating, husbandry of parents and offspring, replacement of breeding stock and provision of special requirements such as caging, nesting material, nest boxes and whelping quarters (whelping refers to breeding dogs).

8.3.1. Criteria for the choice of system

In order to breed laboratory animals successfully, a system has to be devised that takes into account the behaviour and reproductive physiology of the species and the desired genetic characteristics of the young produced.

Examples of the effects of reproductive physiology

Although all the mammals covered in this chapter have an oestrous cycle, there are differences between them that need to be taken into account when devising a breeding system. Some females are able to mate all year round (e.g. mice and rats); others mate only at certain times of the year, in their breeding season (e.g. sheep and ferrets). In most seasonal breeders, it is the female that has a definite breeding season, whereas the male is able to mate all year (e.g. sheep), but, in the ferret, both the male and the female are sexually active only during the breeding season. Some species (e.g. rats and mice) ovulate at a set time in each reproductive cycle; this is called spontaneous ovulation. In others (e.g. rabbits), mating induces (stimulates) ovulation (see **Table 8.3**).

Examples of the effects of behaviour

Behaviour can affect breeding systems in a variety of ways. For instance, female rabbits object to males being present when giving birth in confined spaces. Syrian hamsters will fight if put together for the first time when adults, unless the female is in oestrus, in which case they will mate before fighting. Females of many strains of rat object to other females being present during birth.

Examples of the effects of desired characteristics

Animals may be required that have specific genetic characteristics, which will determine the type of breeding system that must be used; for instance, the foundation stock of an inbred strain must be bred using monogamous pairs. At other times there may be a request for no specific genetic type, in which case harems may be the most appropriate system, as they are cheaper to operate.

Mating arrangements

Table 8.2 describes the arrangements used for breeding laboratory animals. Ratios of males to females in harems are given in the table and when discussing breeding individual species.

Table 8.2. Mating arrangements

Monogamous pairs	Housing one male and one female together, usually throughout their breeding lives. The young remain with the parents until weaned. This method is expensive because it requires more space, labour and materials, and requires as many males as females. However, it does take advantage of postpartum oestrus, pre-weaning losses are low and accurate record-keeping is straightforward.
Harems	Housing one male with two or more females. Harems can be **permanent**, whereby the male and females remain together throughout their breeding lives, or **non-permanent** (also known as **boxing-out systems**), whereby the females are removed for parturition and returned to the harem when the young are weaned In permanent harems, young remain in the harem until they are removed when weaned. Fewer males are required, the method requires less space and maintenance than monogamous pairs and postpartum oestrus can be taken advantage of. Accurate breeding records are more difficult to keep because females may have litters at the same time and the females of some species (e.g. mice) share feeding and rearing duties. Boxing-out is used when it is necessary for the parentage of young to be accurately recorded or for those species that do not like to give birth in the presence of others (e.g. rats). Postpartum mating cannot be used in this system, but there tends to be lower pre-weaning loss because there are fewer animals in the cage and less competition for milk. A small harem of one male and two females is called a trio and may be continuous or discontinuous.
Arranged or observed mating	Taking a selected female in oestrus to a male's cage or pen for mating. She is left either for a specified time or until mating is observed; she is then returned to her own cage. Detailed records may be easily kept. The system is expensive, as it requires more space and labour

Source: Stephen Barnett

8.4. Breeding systems for selected species

8.4.1. Introduction

This section covers breeding common laboratory animals. The techniques described are in common use. However, there are many different strains of laboratory species (there are hundreds of mouse strains alone); each strain will have differences in their genetic make-up, and therefore exhibit different phenotypic characteristics, some of which may affect breeding. Some strains may develop slower than other strains, and so may reach sexual maturity at a later age and some strains may have slightly different gestation periods, and some may not be successful in rearing young (see

Chapter 8.7.4). It is therefore important to obtain as much information as possible about the strain that is being used before beginning to breed them.

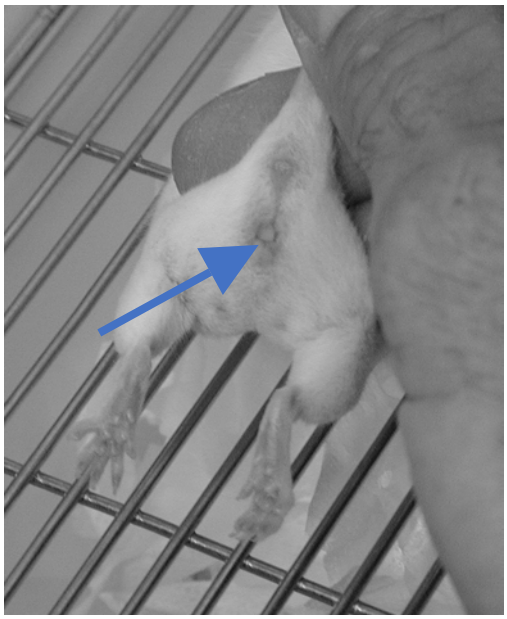
A point that has been made before in this chapter is the importance of not overbreeding. Breeding too many animals leads to a waste of animal life, is unethical and goes against the ethos of the Three Rs. The Jackson Laboratory has published a 'Breeding colony size planning worksheet' that shows how many breeding units must be set up to provide the number of animals required for use. The worksheet is available online to download, free of charge.

8.4.2. The mouse

The oestrous cycle in the mouse lasts for four or five days, and oestrus lasts for approximately 12 hours. Pro-oestrus and oestrus can be identified by their visual appearance, but, if all four stages of the oestrous cycle need to be identified, a vaginal smear is more accurate. Both these methods are described by Byers *et al*. (2012).

Mice can be bred in monogamous pairs, permanent harems or discontinuous harems. They are social animals and, in harems, the females cooperate in feeding and rearing the young. Mating usually takes place at night, so it is not often observed. The presence of a cream-coloured, waxy plug in the vagina of the female in the morning indicates that mating has taken place. The plug remains in place for only a few hours (**Figure 8.1**). Experienced people can identify pregnancy at about seven days of age by palpating the abdomen, but this technique has the potential to damage both mother and pups and should be carried out only after appropriate training. Ultrasound scanners have also been developed for small mammals; in experienced hands, these can be used to identify pregnancy at about seven days (Pallares and Gonzales-Bulnes, 2009). Mice should be provided with a suitable material to build a nest for their young (see **Chapter 6.3**).

Figure 8.1. Vaginal plug in a mouse



Source: Stephen Barnett

Figure 8.2. Inbred mouse CBA/ca (dark); outbred mouse CD-1 of the same age

Source: Roger Francis

Gestation usually lasts 19–21 days, but, when successful postpartum mating occurs, it may lengthen by as much as five or six days due to delayed implantation. The reported average litter size is eight and the average birthweight is 1 gram. Note that these are average figures, and there are strain differences. Most inbred strains will produce smaller litters that will develop more slowly and will be individually smaller when fully grown (**Figure 8.2**).

Figure 8.3. A one- or two-day-old mouse pup showing closed eyes and ears and baldness typical of altricial young

Source: Stephen Barnett

Like rats, hamsters and rabbits, mice are born deaf, blind, hairless and unable to thermoregulate. Newborn animals of this type are called altricial (**Figure 8.3**). Young mice are moved from the parents' cage at 19–21 days to same-sex groups. This ensures that they are removed before the arrival of the next litter, and also prevents unwanted mating.

8.4.3. The rat

The oestrous cycle in the rat takes four or five days to complete. Oestrus lasts approximately 12 hours and usually occurs at night, but mating may be observed during the day.

Rats can be bred in monogamous pairs or harems. It is normal practice to box out pregnant females, as their intolerance of the presence of others when rearing young results in high pre-weaning mortality. The vaginal plugs of rats, which indicate that mating has taken place, are easily seen, but, unlike those of mice, tend to have fallen out by the next morning. If timed mating is required, the rats can be kept in grid-bottomed cages with paper on the tray underneath the grid so that the plugs can be easily seen (**Figure 8.4**). The female must be returned to a solid-bottomed cage with suitable nesting material as soon as possible after mating has been confirmed.

The average gestation period is 21–23 days. Parturition usually takes place in the early morning. Weaning and the recurrence of oestrus after giving birth are the same as for mice.

Figure 8.4. Rat vaginal plugs

Source: Roger Francis

8.4.4. The Syrian hamster

The female hamster has an oestrous cycle of four or five days. Oestrus lasts for approximately 12 hours. The presence of a cream-coloured, stringy discharge, with a distinctive smell, from the vagina (**Figure 8.5**), called the post-ovulatory discharge, signals the end of oestrus. The next oestrus will occur three days after the discharge is noted. If the female's back is stroked, she will raise her tail and arch her back (behaviour known as 'lordosis') when she is in oestrus. Copulation plugs are not seen.

Figure 8.5. Post-ovulatory discharge

Source: Stephen Barnett

Syrian hamsters can be bred from trios or monogamous pairs, provided that they are set up at weaning age. If it is necessary to mate hamsters for the first time when they are adults, arranged mating must be used. In this case the female is taken to the male's cage and mating is observed. If mating does not occur, the female may act aggressively to the male and must be removed immediately.

Hamsters have the shortest gestation period of the common laboratory animals, at 16 days. Postpartum oestrus occurs, but mating is unlikely to be successful at this time. The oestrous cycle will resume at the end of lactation. The young are weaned by 21 days.

A minimum of 12 hours of light and room temperatures of 21–24°C will prevent hamsters from hibernating in winter and minimise the seasonal effect on breeding performance.

8.4.5. The guinea pig

The guinea pig has an oestrous cycle that lasts for 14 days and an oestrus period that lasts approximately 24 hours. Female guinea pigs have a hymen (a membrane across the vaginal opening), which regresses spontaneously a day or two before oestrus and regrows after each oestrus.

Guinea pigs are usually bred from permanent harems with a ratio of 1 male to up to 10 females, although, in practice, enclosure size will dictate lower ratios. As the gestation period is long and the lactation period is short, postpartum mating is not likely to result in delayed implantation. Young guinea pigs will often feed from any lactating female. Boxing-out of pregnant animals from a harem, breeding in monogamous pairs and arranged mating are usually carried out only when specifically required by a study, because they are expensive in terms of space, labour and materials.

Mating takes place during the day or night; therefore, it is more often observed than in the other small species. Vaginal plugs do occur, but are fairly difficult to find.

Owing to the long gestation period of 65–72 days, the young are very large in the later stages of pregnancy, and the female must be very well supported if it is necessary to handle her. During birth, the two halves of the female's pelvis have to be able to open wide enough to allow the young through. The point where the pelvis is joined, called the pubic symphysis, is held together by ligaments, which relax under the influence of hormones released in late pregnancy. If a female is not mated by the

time she reaches seven months of age, the pubic symphysis ossifies (it fuses with bone) and is not able to open to let the young through. It is important not to attempt to breed from a female guinea pig for the first time if she is over the age of seven months.

When they are born, the young are fully furred and are able to thermoregulate, move about and eat soft solid food. Young with these characteristics are called precocial (**Figure 8.6**). Nesting material is not necessary because the young are advanced at birth, but all guinea pigs benefit nutritionally and environmentally from the presence of hay in their cages. The lactation period is short, about 14 days, which coincides with the weaning age. Oestrus will recur post partum and again at the end of lactation.

Figure 8.6. Young guinea pigs, fully furred and active, typical of a precocial species

Source: Stephen Barnett

8.4.6. The rabbit

In the rabbit, oestrus lasts for several days, for several weeks or until the female is mated. The readiness of the female to mate is indicated by a reddened and slightly enlarged vulva. Does (females) can be aggressive to the male when kept in confined conditions; for this reason, they are bred only from arranged, observed mating. When the enlarged vulva is noticed, the doe is taken to the buck, mating is observed and the animals are separated when mating has occurred. Ovulation is induced by mating; it is advisable to repeat the mating 24 hours later to ensure successful fertilisation. The gestation period of large breeds, such as the New Zealand white rabbit, is 30–32 days; smaller breeds, such as the Dutch rabbit, have gestation periods of 28–30 days. Rabbits have a postpartum oestrus, but re-mating at this time is not recommended. Oestrus will return at about the fourth or fifth week of lactation.

When parturition is imminent, the female will strip the fur from her underbelly to supplement the nesting material supplied in the nest box or nesting area (**Figure 8.7**). In addition to providing a comfortable nest, stripping fur allows the young to find the nipples with greater ease, and it enables heat to be transferred to the young more readily. It is advisable not to interfere with very young litters, as rabbits tend to reject or even kill their young if they are handled. Females feed their young only once a day. Weaning is complete by 5–8 weeks.

Figure 8.7. Young rabbits in hay nest lined with fur from the mother

Source: Stephen Barnett

Although there is no definite breeding season, there is a considerable reduction in production in the autumn and winter months, in spite of any environmental control.

8.4.7. The ferret

Ferrets are usually bred from arranged, observed mating.

The vulva of the female is grossly enlarged and red to purple in colour when she is in oestrus; when not in oestrus, the vulva is smaller and pale. Both male (hobs) and female (jills) ferrets have a definite breeding season: sexual activity occurs only from March to July, although the season may be extended by the alteration of lighting regimes (early or late wintering). Oestrus is prolonged in the absence of the male.

The female is taken to the male's pen about 10 days after her vulva starts to swell. They are left together for 24–48 hours. During courtship and mating, the male drags the female around the pen. Tying occurs (where the penis becomes locked in the vagina) and mating may be prolonged. Ovulation is induced by mating.

The average gestation period is 42 days. Oestrus will recur at the end of lactation if it is still within the breeding season; hence, it is possible for a female ferret to have two litters in one year (**Figure 8.8**). Weaning is complete by 6–8 weeks. Ferrets have altricial young, so they must be provided with suitable nesting material to keep them warm when first born.

Figure 8.8. Litter of ferrets

Source: Marshalls UK

8.4.8. The cat

Cats are seasonal breeders in natural light conditions, but will breed all year round provided that they are exposed to 12–14 hours of light a day. They have an oestrous cycle of 14–21 days, and oestrus lasts for 3–10 days. Cats exhibit induced ovulation. They are bred by arranged mating or in large harems.

A female cat (or queen) in oestrus is easy to recognise as she wails, rolls and exhibits lordosis (arching of her back). With arranged mating, the female is taken to the tom (male) and they are left together for 24–48 hours. During mating the male grips the female around her body with his forelegs and grips her neck with his teeth. Mating is rapid; one thrusting movement stimulates ejaculation. At the end of mating, as the male withdraws his penis, the female gives a loud shriek and exhibits post-mating behaviours that involve rolling and licking her vulva. Humans or males that attempt to approach her during this time risk being attacked. After 20–30 minutes she will allow the male to come near and the mating process will be repeated. The sensation caused by the withdrawal of the penis provides the stimulus to induce ovulation.

The average gestation period is 63–65 days and the females are boxed out into a littering cage about one week before parturition. Kittens are born fully furred but are deaf, blind and completely dependent on their mother. Young of this type are called semi-altricial. Development is rapid and the young are fully weaned by 6–8 weeks. Oestrus will usually recur during the fourth week of lactation.

8.4.9. The dog

Pro-oestrus may be detected in the female (the bitch) by observing a bloodstained discharge from the vulva. The vulva swells and the discharge becomes straw-coloured as the bitch enters oestrus. Oestrus occurs about 10 days after the first discharge is noted and lasts for 7–10 days. Dogs can be bred in harems and by arranged mating. Where arranged mating is practised, it is usual to repeat the procedure three times on separate days.

Like most mammals, bitches will allow themselves to be mated only when they are in oestrus; they are said to 'stand' for mating, or to be in 'standing heat'.

Mating in the dog can be prolonged (20-30 minutes) and a very large amount of ejaculate is produced. Initially a sperm rich fraction is released and this is followed by fluid from the prostate gland which provides protection and a medium for the sperm to swim in. Mating starts with the dog mounting the female from the rear, during the process the animals become tied or locked together. Tying results from the swelling of the glandular portion of the penis within the vagina. When this occurs the dog changes its position by dropping to one side of the female so they are back to back (**Figure 8.9**).

Figure 8.9. Beagles tied during mating

Source: Source Pfizer UK

The normal oestrous cycle of the female lasts 6–7 months. Pregnancy has little effect on the length of the cycle. More bitches come into oestrus in the spring and autumn than in the summer and winter. The average gestation period is 63–65 days.

Pups, like kittens, are born fully furred, but deaf and blind and totally dependent on their mother (semi-altricial). They are fully weaned by eight weeks of age (**Figure 8.10**).

Figure 8.10. Beagle pup in a Vetbed nest

Source: Source Pfizer UK

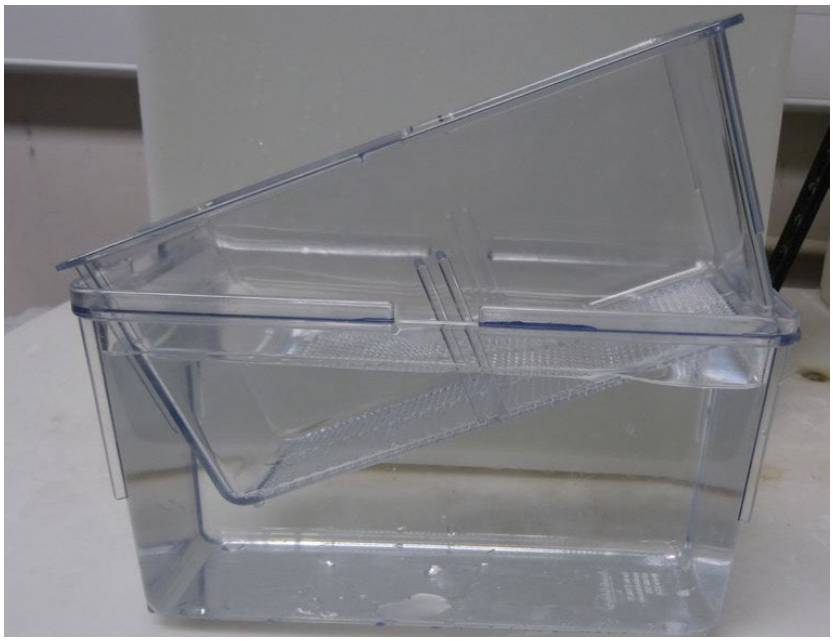
8.5. Example of a non-mammalian species

8.5.1. Notes on zebrafish health, by Nicola Goodwin

Once zebrafish have reached sexual maturity, they are capable of spawning on a daily basis. To ensure optimal egg quality and quantity, it is recommended to provide a resting period of at least one week between breeding sessions. Egg production and spawning is affected by the health, size and age of the fish, and by the diet and light cycle provided. To ensure optimal reproduction, the light cycle should be controlled to 14 hours of light and 10 hours of dark.

Zebrafish are commonly bred within pairs (one male and one female) or small groups (three males and three females) using small static breeding tanks (no water flow). The breeding tank should contain a meshed insert that the eggs pass through when they are spawned, preventing the adult fish from eating them. To increase egg production, the tank insert can be tilted, creating a shallow area and, therefore, a more natural breeding environment. However, it is important to ensure that the total water volume available for the fish to swim is maintained at a minimum of one litre, as required by legislation (**Figure 8.11**).

Figure 8.11. Mesh insert in a production tank (the minimum water volume must be at least 1 litre)



Source: Nicola Goodwin

Ovulation typically occurs just prior to dawn, with spawning commencing within the first few hours of light. During spawning the female can lay several hundred eggs, under optimal conditions.

Following spawning, the fish should be placed back in their home tanks and the eggs collected from the breeding tank. Zebrafish eggs are relatively soft and therefore should be carefully removed from the tank. This is typically performed by pouring the eggs from the breeding tank into a soft meshed tea strainer. System water should pass through the strainer while it contains the eggs. The eggs should then be rinsed with egg water (a mix of salt, methylene blue and reverse osmosis water) before being placed in a petri dish that also contains egg water. Within 24 hours of collection, the non-viable eggs should be removed and viable eggs split into fewer than 50 per dish.

Zebrafish larvae will hatch from the eggs at approximately three days post fertilisation, and inflate their swim bladders at approximately five days post fertilisation. During this time no food is provided, as the fry have a yolk sac to provide nutrition. This timescale can change depending on the temperature of the incubator they are held in (cooler temperatures slow development). Once capable of independent feeding (when the swim bladder has inflated), the fry should be removed from the incubator and placed within the aquatics system.

8.6. Genetically defined laboratory animals

A genetically defined animal is one that has been bred so that the arrangement of its genes is known, for example inbred, random bred and outbred.

8.6.1. Inbreeding

Inbreeding occurs when closely related animals are mated together. It occurs intentionally when an inbred strain is being developed but it can also happen unintentionally in non-inbred colonies where management is poor. In closed colonies it is inevitable that it will occur, and eventually the colony will have to be opened to allow in new genetic material. The number of generations it takes before that happens depends on the size of the colony; minimal inbreeding techniques must be used to delay it as long as possible in non-inbred colonies (see **Chapter 8.6.2 'Outbreeding'**).

Breeding inbred strains

An inbred strain is produced as the result of 20 consecutive generations of brother × sister or youngest parent × offspring matings. Inbred strains have certain characteristics that make them valuable experimental animals. The following are the two main ones:

— they are isogenic, which means that all individuals within the strain are genetically identical;

— they are homozygous, meaning that over 98.6% of the two genes (one copy from the male parent and one from the female parent) for each characteristic are identical.

Other characteristics of inbred strains follow from these two:

— the strains are genetically stable (because of the high degree of homozygosity);

— they are more phenotypically uniform than non-inbred animals (because they are isogenic);

— each inbred strain is unique in that only one strain has that particular combination of genes;

— strains can be identified using genetic monitoring techniques.

Using inbred strains for scientific study eliminates one of the major influences on experimental results, genetic variability. This means that statistically meaningful experimental results are gained using fewer animals.

Production of inbred strains

Foundation Stock

Inbred strains start from pairing one male and one female. The offspring produced from this mating are then paired (brother × sister); this is called the F1 generation, and the offspring from the F1 generation are paired to produce the F2 generation, and so on. Only one brother × sister pair will be chosen to produce

the strain, but, as some pairings can be expected to die out due to inbreeding depression, as many brother × sister pairings as possible are set up at each generation. Accurate records are kept of the breeding performance, in the form of a pedigree chart, of these pairs. After every three or four generations, the breeding performance of all pairs is assessed and the line that is most productive is chosen as the main line of descent. The other surviving lines are stopped from breeding.

This goes on for 20 consecutive generations, after which the strain can be said to be inbred.

Producing a new inbred strain is not a common thing to be asked to do. A more usual requirement is to maintain an established inbred strain. The same technique that has been described above to establish a new inbred strain has to be used to maintain the main line of descent, also called the foundation stock.

Expansion colony

The foundation stock maintains the inbred strain, but does not produce many animals for experimental use. Therefore, the number of animals produced has to be increased. This is achieved by removing animals produced in the foundation colony, not required for breeding, to an expansion colony. In the expansion colony they can be used for experimentation or can be used for breeding to increase the number of animals. However, breeding must not go on for more than three generations after they have left the primary colony. Up to three generations will not affect the inbred status of the animals, but a greater number of generations away from the common ancestor in the primary colony will risk a genetic separation from it and the animals will no longer be considered inbred.

Inbreeding depression

Most genes that code for harmful characteristics (deleterious genes) are recessive. That means they can effect the phenotype of the animal only if the deleterious gene is homozygous. In non-inbred animals this situation rarely arises because deleterious genes, if present, are masked by dominant 'normal' genes. In the development of inbred strains, the genes become progressively more homozygous, so deleterious genes are more likely to be expressed. This often leads to inbreeding depression, characterised by:

— small litter sizes;

— small pup sizes;

— slow growth rates;

— high pre-weaning mortality;

— long litter intervals;

— increased susceptibility to disease and environmental change.

Hybrid vigour

For reasons explained above, inbred strains often suffer from inbreeding depression. Experimental factors may require animals that are isogenic, but with the vigour associated with non-inbred animals. These characteristics can be produced by mating animals from two inbred strains and using their offspring for experiments. The first generation of such matings (the F1 hybrids) will be heterozygous, but isogenic. Only the first generation of offspring hybrids can be used, as subsequent generations will cease to be isogenic.

Genetic drift

Genetic drift refers to genetic differences between the current generation of an inbred strain and previous generations. These differences are caused by spontaneous and random mistakes that can occur when DNA is replicating. The effect that these mistakes (mutations) have may be observable, such as a colour change or obvious change in productivity, or it may be more subtle and appear only at a later stage, for instance when unexpected experimental results become apparent. Routine genetic monitoring will not necessarily pick up genetic drift, as genetic monitoring looks for genetic markers; it does not look at the whole genome. Complete genetic screening is possible, but is very expensive, so it is often carried out only annually or every six months.

Genetic drift happens to all inbred strains, so one strain maintained at two different centres will diverge even if the original stocks were obtained from the same supplier. This obviously has implications for collaborative work between research groups. It cannot be prevented, but there are ways to reduce its effects, for instance backcrossing to the stock from the original strain (for more information, see Low-Marchelli, 2017.

Genetic divergence can occur for reasons other than genetic drift, mainly associated with human error, for example incorrect pairing or leaving animals breeding in the expansion colony for too long.

Identification of genetic difference

The productivity of inbred strains is poor compared with that of hybrids (see inbreeding depression above); therefore, if an inbred colony suddenly increases its productivity, or the size of the pups increases, it indicates that the strain has been contaminated. Colony productivity will show up in breeding records. A coat colour change, even if it is only a small area, also indicates a genetic change.

Regular genetic monitoring is used to ensure that inbred strains are breeding true. Tissue samples are taken from the animals, usually from the ear, and are analysed to see if they contain the series of genetic markers unique to that strain; however, as mentioned previously, routine genetic screening may not pick up genetic drift. Routine screening looks to see that specific markers are present; it does not look for everything.

8.6.2. Non-inbreeding techniques

Random breeding

Random breeding involves selecting animals for mating without any regard to their relationship. Other than ensuring that the animals are from the required strain, healthy and of the opposite sex, there are no selection criteria. Theoretically, a random selection procedure should be used that involves random number tables or random number generators (small computer apps).

Some animals set up by this method could be closely related, even brother and sister; others will be distantly related. However, at each generation, the situation will change and separate lines of descent are prevented from being established. The degree of inbreeding differs at each generation.

Outbreeding

Outbreeding is the active avoidance of inbreeding. Only distantly related animals are paired. If the colony is an open one, new animals from other colonies can be introduced to prevent inbreeding. In

closed colonies this is not possible, and eventually a degree of inbreeding is inevitable. Minimal inbreeding techniques can be used to minimise inbreeding.

Minimal inbreeding requires that monogamous pairs are used because, if a harem is used, and one male mates with more than one female, all the offspring will be related. It also requires that each pair in the colony contributes the same number of offspring to the next generation of breeders. Systems have been devised to make the avoidance of inbreeding simple to arrange. The most straightforward of these systems is as follows: each breeding pair is given a number and the offspring of the pair keep the number until they are selected for breeding. Pairing then follows the following pattern:

— offspring from pair 1 are paired with offspring from pair 2; they make new pair 1;

— offspring from pair 3 are paired with offspring from pair 4; they make new pair 2, and so on.

This can be summarised as follows:

$1 \times 2 = 1$, $3 \times 4 = 2$, $5 \times 6 = 3$, $7 \times 8 = 4$.

8.7. Specific genetic models

8.7.1. Co-isogenic strains

Co-isogenic strains are two strains that are genetically identical except for one gene. This can arise as a single mutation in an inbred strain that has then been maintained by breeding.

8.7.2. Congenic strains

These are two strains that are identical except for a short segment of chromosome bearing a gene of interest. They are produced when a gene of interest is identified in an animal. In order to study the effects of the gene, it is useful to breed it into an established inbred strain. This is done by mating an animal with the gene (called the donor) with an animal from a inbred strain. Offspring that carry the gene are identified and backcrossed to the inbred strain. This is repeated for 15 backcrossed generations. The result is a strain that carries the gene and a strain that is identical to it except that it does not carry the gene. The two strains can be compared to see the effect that the gene has on the animal or how it effects experimental treatments. If the gene of interest is a recessive one, progeny testing (backcrossing offspring to the donor animals) has to be done between each generation to identify the animals carrying the gene.

8.7.3. Recombinant inbred strains

These are produced by mating a male from one inbred strain and a female from another inbred strain and pairing their offspring in the same way as in an inbred strain; each of the pairings is continued for 20 consecutive generations. After this time a number of inbred strains will have been produced, each containing a set of genes that have originated from the two original inbred strains. When used in conjunction, recombinant inbred strains can be used to identify characteristics that are controlled by single genes or many genes (polygenic) and can be used for gene mapping studies.

8.7.4. Genetic mutants

Some strains of animal carry naturally occurring genetic mutations that cause them to develop diseases that are similar to conditions seen in humans (e.g. diabetic rat, obese mouse) or make them useful in the study of physiological systems (e.g. the athymic (nude) mouse and the severe combined immunodeficient mouse used in immunological studies). Animals that are homozygous for the characteristic are often poor breeders, so it is necessary to breed from animals that are heterozygous for the characteristic.

In the case of nude mice, the homozygous female has difficulty in rearing her young, so pre-weaning mortality is high. Females carrying one copy of the nude gene (heterozygous nudes) have a normal phenotype. It is therefore common practice to mate heterozygous females with homozygous males to produce litters that, on average, will be 50% homozygous and 50% heterozygous animals (**Figure 8.12**).

Figure 8.12. Breeding box of nude mice. A = heterozygous pups (hairy); B = homozygous pups; C = heterozygous female (hairy); D = homozygous male (nude)

Source: Roger Francis

Successful breeding of animals with specific mutations do not always follow this pattern; therefore, different breeding arrangements will need to be used. In the case of diabetic rats, for instance, homozygous diabetics can be bred from, provided that their diabetes is controlled by medication.

8.8. Breeding records and productivity measurements

Breeding colonies must be carefully monitored to ensure that acceptable breeding performance is maintained. Productivity in the colony has obvious economic importance, but, more importantly, it is a gauge of the health and welfare of the animals. The appearance of pathogenic organisms in the animal breeding area, loss of environmental control, a poor batch of diet or even an unsympathetic or poorly trained animal technician can affect the performance of the breeding colony, leading to reduced fertility, an increase in pre- and post-weaning mortality rates and/or reduction in growth rates. These effects will often precede any obvious clinical signs of ill health.

In addition to maintaining records of the source, use and final disposal of animals, the following information must be kept:

— colony size,

— individual performance per breeding female (in some case for individual males),

— total output of the colony,

— litter size,

— number of litters in a given period,

— pre-weaning and post-weaning mortality rates.

Not only must records be kept but they must be regularly monitored so that any change is noted and quickly investigated.

Colony management computer programs that record and analyse all breeding data are available from specialist companies. As with all computer programs, they record only what is fed into them, so accurate computer skills are required to ensure that the correct data are entered. Full analysis of production performance is available. The programs allow users to set expected production figures, such as litter intervals and litter sizes, and will produce alerts if these are not as expected.

8.9. Selection of breeding stock

All potential breeding animals must be inspected to ensure that they are fit enough to breed. The selection of breeders for inbred, random bred and outbred strains has been covered in **Chapter 8.6**.

In larger animals, for example dogs and non-human primates, veterinary inspections may be carried out before they are used for breeding and between each birth to ensure that they are fit enough to produce another litter.

In other systems, breeding stock are selected from the information available in their records and from the appearance of the individuals. Delaying the selection of the next generation of breeders until the second or subsequent litters from a female enables more information about her breeding performance to be available, and therefore more reliable decisions to be made.

The ideal future breeding animal would be:

— free from visible (or detectable) unwanted abnormalities or characteristics;

— from a 'good litter' in terms of:

  - number of offspring,
  - size of offspring,
  - even sex distribution,
  - acceptable litter interval;

— from parents with good previous records;

— from parents of good temperament and good nurturing ability.

When finally selecting breeders, particular attention should be paid to the condition of the genitalia (e.g. well-developed testicles in males, clean appearance to the vulva in females) and to characteristics such as the appropriate number and arrangement of teats.

8.10. Weaning

In small rodents (mice, rats and Syrian hamsters) it is usual to complete weaning by removing the young from their dam on a specific day in accordance with a routine. This is done at about three weeks, but, with inbred strains or GAAs that exhibit poor growth, weaning may be delayed if the female is not pregnant.

Guinea pigs are often weaned when they achieve a certain body weight (e.g. 180–200 g, which they reach at about 14 days of age).

Rabbits are usually weaned by age, at 6–8 weeks. Ferrets, cats and dogs are also usually weaned by 6–8 weeks of age, but each litter will be considered individually.

8.11. Pre-weaning mortality

The reproductive mechanism is not faultless, and a small percentage of deformed, small or unthrifty animals (runts) will occur in any colony; however, they may not be seen, as parents often eat such young. These runts, and some offspring that are born apparently normal, may not survive until weaning due to disease, poor nurturing, competition for food, or deficiency in the nest or the general environment.

An increase in the pre-weaning mortality rate of a breeding colony indicates underlying health and welfare problems within the colony.

8.12. Economic breeding life

In the smaller laboratory species, it is usual to replace breeding stock as follows:

— rats, mice and hamsters: after about 6 litters;

— guinea pigs: after 6 to 8 litters;

— rabbits: after 10 to 12 litters or, in a small colony, depending on the breeding performance of individuals.

Although the breeding performance of males does not diminish at the same rate as that of females, it is usual, in the smaller species, to replace the males at the same intervals as the females.

In the larger laboratory species (e g. ferrets, cats and dogs), the economic breeding life will depend on the breeding performance of the individual animals. Both males and females are given veterinary checks after each litter to ensure that they are fit enough to produce another.

8.13. Productivity

The assessment of the productivity of a particular female is usually stated as the number of offspring weaned per week (or month or year). Such calculations are influenced by the number in the litters at weaning and the interval between litters (see **Table 8.1**, glossary of breeding terms).

Table 8.3 shows typical average litter intervals, litter sizes and the productivity of the common laboratory species, but note that this information is a guide only; there are variations between different strains and colonies.

Table 8.3. Summary of breeding data of common laboratory species

Species	Breeding Season	Duration of oestrus	Type of ovulation	Length of oestrous cycle	Gestation period	Average litter size weaned	Recurrence of oestus post partum	Litter Interval or litters / year	Economic breeding life
Mouse	All year	10-12 hours	spontaneous	4-5 days	21 days	8	Immediate then at end of lactation	4-5 weeks	6 litters 6 months
Rat	All year	10-12 hours	spontaneous	4-5 days	21 days	10	Immediate then at end of lactation	6-7 weeks	6 litters 8 months
Syrian hamster	All year[7]	12 hours	spontaneous	4 days	16 days	6	Immediate then at end of lactation	7 weeks	6 litters 8 months
Guinea pig	All year	10-20 hours	spontaneous	14 days	65-72 days	3.5	Immediate then at end of lactation	4-5 litters	10-12 litters 2-3 years
Rabbit	All year	Extensive if not mated	induced	Extensive if not mated	Small breeds 28 days Large breeds 31 days	7	Immediate[8] then 3-4th week of lactation	4-5 litters	10-12 litters 2-3 years
Ferret	March -July	Extensive if not mated	Induced	Continuous if not mated	42 days	8	End of lactation	2 litters	6-8 litters over several years
Cat	All year[9]	7-10 days	induced	14-21 days	63 days	4	4th week of lactation	2 litters[10]	Determined by veterinary inspection
Dog	All year	5 days	spontaneous	6-7 months	63 days	4	Next oestrus		

NB: All figures are averages and there are strain differences within species.
Source: Stephen Barnett (Adapted from P J Kelly, K G Millican and Pam Organ)

[7] In laboratory conditions.
[8] Not used in laboratory breeding
[9] In laboratory conditions.
[10]Determined by veterinary assessment to maintain good welfare.

8.14. Physical development of young animals

8.14.1. Mice and rats

Mice and rats are born into well-formed nests (**Figure 8.13**). They are naked and their eyelids are closed, but they are able to move sufficiently to find a teat and can squeak to attract the attention of their mother. Communication between mother and young is often in ultrasound. The ages of mice and rats may be estimated from their physical development. **Table 8.3** details stages of development in average outbred strains of mice and rats, and **Table 8.4** gives average birthweights, ages and weights at weaning and first mating, and average weights when adult. Considerable variations occur between different strains of animals; for instance, Wistar rats are smaller than Sprague Dawley rats, and inbred CBA mice are larger than inbred DBA mice. Inbred strains will not only be smaller than outbred strains, but also develop at a slower rate. These variations should be noted when interpreting these tables and other tables and charts in this book.

Figure 8.13. Rat nest

Source: IAT

Table 8.4. Stages of physical development in outbred mice and rats

Newborn	The eyelids are closed. Pups are naked except for short whiskers. They are bright pink with translucent skin, which enables the abdominal organs to be seen. If the pup has suckled, the milk is visible in the stomach. The ear flaps (pinnae) are small and are tight to the snub-nosed head (**Figure 8.14**)
2–3 days	Whiskers are longer and more obvious. The skin is a duller pink and opaque and the internal organs are no longer as visible. The pinnae start to lift away from the sides of the head
About 5 days	Coloured strains begin to show skin pigmentation
8–10 days	Most of the body is covered by short, fine fur of its final colour
About 10 days	The incisors erupt and the teats of young females become obvious because they are not covered by fur (**Figure 8.15**)
12–14 days	Eyelids are open. The snout is longer and pups are very mobile when disturbed
14–16 days	They are well covered with fur. They spend more time out of the nest and begin to eat the crumbs of diet below the hopper
17–18 days	The pinnae are larger and stand away from the head. Fur fully covers the underbelly
19–23 days	The first and second pair of molars erupt. At this stage, the pups are self-sufficient and weaning can be completed (**Figure 8.16**)
Mice at 5 weeks / rats at 7 weeks	They reach sexual maturity, the vagina is open and the testes have descended into the scrotal sacs. The third pair of molars erupt

Source: P J Kelly, K Millican and Pam Organ

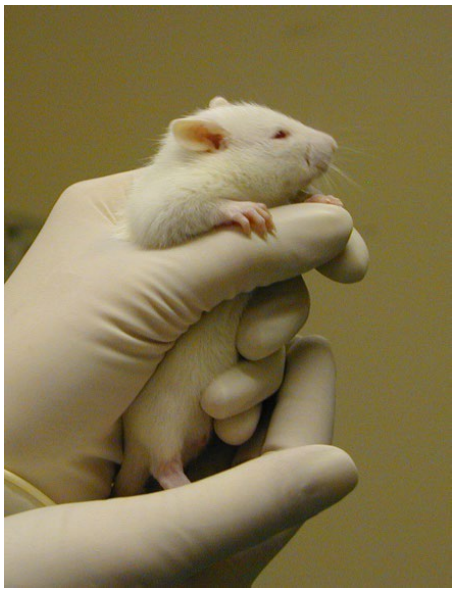
Figure 8.14. A one-day-old rat. Eyes closed, pinnae close to the head, no hair on body

Source: Stephen Barnett

Figure 8.15. An 11-day-old rat. Eyes closed, pinnae separating from head, body covered with short hair

Source: Stephen Barnett

Figure 8.16. A 19-day-old rat. Fully furred, ears separate from head



Source: Stephen Barnett

8.14.2. Syrian hamsters

Like mice and rats, Syrian hamsters are born into well-formed nests. Their physical development is similar to that of the mouse.

8.14.3. Guinea pigs

Guinea pigs do not make proper nests, as the young are well developed when born. At birth they are already covered with short fur, their eyelids are open and they are capable of eating solid food, although the female provides milk for 14 days. They are fully mobile within a few hours and have the appearance of small adults within 14 days of birth.

8.14.4. Rabbits

Rabbits construct nests with any material available to them. The female lines the nest with fur taken from her abdomen. Unless absolutely necessary, young should not be disturbed until they start to leave the nest at about two to three weeks of age. The female may reject young that have been touched. The kits are born hairless and with their eyes closed, but are able to move a little for feeding. By seven days they will have doubled their birthweight, will have a well-developed coat and will be able to move more easily (**Figure 8.17**). By 10 days of age, the eyes are open and the ears are functioning. Between 15 and 21 days, they leave the nest and feed independently. Weaning is completed by 5–8 weeks.

Figure 8.17. A 9- to 10-day-old rabbit

Source: Stephen Barnett

8.14.5. Carnivores (cats, dogs and ferrets)

A nest area is normally provided for these species. The young are born with fur, but with eyelids closed. They can crawl to find a teat and can vocalise to attract their mothers' attention. The eyelids open at about two weeks. The young are totally dependent on milk for about the first four weeks of life and will eat soft solids before learning to lap (**Figure 8.18** and **Figure 8.19**). They are all usually weaned by 6–8 weeks of age.

Figure 8.18. A 25-day-old kitten. Eyes open, but ears still not fully separated

Source: Stephen Barnett

Figure 8.19. A 7-day-old beagle puppy

Source: Pfizer UK

Table 8.5. Approximate ages and weights of outbred animals at different stages of development

Animals	Birthweight	Weaning age	Weaning weight	Sex	Age at first mating	Weight at first mating	Adult body weight
Outbred mice	1–1.5 g	19-21 days	10-12 g	M	6 weeks	20 g	45 g
				F	6 weeks	20 g	40 g
Outbred rats	4–7 g	19-21 days	40-60 g	M	12 weeks	200 g	800 g
				F	10–11 weeks	150 g	600 g
Syrian hamsters	1.5–2.5 g	21 days	35-45 g	M	6 weeks	80 g	120 g
				F	6 weeks	80 g	130 g
Outbred guinea pigs	50–100 g[11]	14 days	180-200 g	M	4 months	500 g	1 kg
				F	3 months	450 g	800 g
Dutch rabbits	30–50 g	5-8 weeks	300-500 g	M	8 months	2.5 kg	3.5 kg
				F	6 months	2.0 kg	2.5 kg
New Zealand white rabbits	60–80 g	5-8 weeks	1-1.5 kg	M	9–10 months	3–3.5 kg	6 kg
				F	7–8 months	2.5–3 kg	5 kg
Ferrets	10 g	6-8 weeks	300-400 g	M	12 months[12]	700–800 g	1–2kg[13]
				F	9–12 months[14]	700–800 g	0.85–1 kg
Cats	120 g	6-8 weeks	0.5-1 kg	M	12 months	3 kg	5 kg
				F	9 months	2.5 kg	3.5 kg
Dogs (beagle)	400 g	6-8 weeks	1-1.5 kg	M	18 months	12 kg	14–16 kg
				F	14 months[15]	10–12 kg	10–12 kg

Source: P J Kelly, K Millican and Pam Organ

[11] Dependent on litter size.
[12] First season.
[13] There is a marked seasonal weight variation with both sexes.
[14] First season.
[15] Second oestrus.

References

Byers, S. L., Wiles, M. V., Dunn, S. L. and Taft, R. A. (2012), 'Mouse oestrous cycle identification tool and images', *PLOS One*, Vol. 7, No 4, e35538, https://doi.org/10.1371/journal.pone.0035538.

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, OJ L 276, 20.10.2010, p. 33, ELI: https://eur-lex.europa.eu/eli/dir/2010/63/oj/eng.

Low-Marchelli J (2017), 'Strategies to minimize genetic drift and Maximise experimental reproducibility in mouse research.' Charles River Laboratories International Inc. https://animalab.eu/knowledge-base/strategies-to-minimize-genetic-drift-and-maximize-experimental-reproducibility-in-mouse-research

Pallares, P. and Gonzalez-Bulnes, A. (2009), 'Use of ultrasound imaging for early diagnosis of pregnancy and determination of litter size in the mouse', *Laboratory Animals*, Vol. 43, No 1, pp. 91–95, https://doi.org/10.1258/la.2008.007139.

The Jackson Laboratory, 'Breeding colony size planning worksheet', The Jackson Laboratory website, https://media.jax.org/m/468eb6d3f89b171a/original/breeding-colony-size-planning-work-sheet.pdf.

Further reading

Barnett, S. W. (2007), 'The supply and production of protected animals', in: Barnett, S. W. (ed.), Manual of Laboratory Animal Technology, pp 1-12. Blackwell Publishing, Oxford.

Festing, M.F.W. and Lutz C. (2010), 'Introduction to laboratory animal genetics', in: Hubrect, R and Kirkwood, J. (eds), The UFAW handbook on the care and management of laboratory and other research animals, 8th edition, pp 37-60. Wiley-Blackwell.

Francis, R. (2010), 'Refinement in in-house animal production and breeding', in: Hubrect, R. and Kirkwood, J. (eds), The UFAW handbook on the care and management of laboratory and other research animals, 8th edition, pp 163-168. Wiley-Blackwell.

Contribution of this chapter to the Three Rs

This chapter supports the Three Rs by highlighting the following:

— breeding only the animals that are needed prevents overbreeding and the need to kill excess animals;

— producing and rearing young satisfies several of the needs of animals mentioned in **Section 1**;

— breeding records provide not only an indication of productivity, but also early indication of disease;

— when animals do have to be killed (e.g. old breeders), collect tissues for use in relative replacement techniques;

— a knowledge of physiology, behaviour and basic genetics ensures that the appropriate breeding system is used, thereby reducing the risk of pre-weaning loss.

Self-assessment questions

1. Outline the difference between an inbred mouse and an outbred mouse.

2. Describe two ways in which oestrus can be identified in a mouse.
3. A rabbit is described as having induced (or stimulated) ovulation. What does this mean?
4. What is meant by economic breeding life?
5. What do the following terms mean?
    (a) Altricial.
    (b) Precocial.
    (c) Semi-altricial.

9. Genetically altered animals

After reading this chapter, you should be able to outline how GAAs can be used for scientific research. You should also understand the importance of careful monitoring of GAAs (learning outcome 4.11).

The chapter also gives an overview of genome editing.

9.1. Introduction

This chapter summarises the use of, and special care that must be provided for, Genetically Altered Animals (GAAs). Inbred strains and mutant strains are included in the definition of GAA; the breeding of these is covered in Chapter 8. The term 'GAA' also includes animals whose genome has been altered by genetic editing techniques. The potential of the altered genome to cause the animal problems is great; consequently, a programme of careful welfare monitoring must be in place to ensure that problems are ameliorated.

Directive 2010/63/EU (the Directive) regulates the creation and breeding of GAAs; **Chapter 1.3.6** explains this further.

This chapter gives an overview of how GAAs are used in scientific procedures; it then provides an introduction to genome editing in mice and finishes by covering the monitoring of GAAs.

9.2. Uses for genetically altered animals

GAAs are created when a modification of a gene or genes allows the study of their function and how they interact with other genes. Genetic alteration can also encompass the introduction of a new gene, which is then called a transgene.

Genetically altered mice are useful in the study of many diseases that are inherited or partially inherited as a result of faults in an individual's genetic code. Some serious, potentially fatal illnesses, such as sickle cell disease and cystic fibrosis, are caused solely by having faulty genes. Some long-term illnesses, such as diabetes and rheumatoid arthritis, have many causes that include faulty genes, combined with other factors such as infection.

9.3. Genome editing in the mouse: an introductory overview

The mouse is a particularly valuable model system for research into mammalian biology. Importantly, the mouse has many useful similarities to humans. This includes a high degree of similarity in genetic make-up and basic physiological processes. For well over 50 years it has also been possible to generate inbred strains that are essentially genetically identical. By making very precise changes in the genome of inbred mice, it becomes possible to study the effects of genetic variants that are thought to underlie human disease without the genetic variability of a natural population. As a result of this, at present, the vast majority of mammals that have been genetically modified for scientific study have been inbred mouse strains. Given this, this chapter will focus on the development of methods to make genetically modified mice. However, many of the basic principles of genome editing that are discussed in this chapter will apply to the generation of other GAAs. **Table 9.1** provides a glossary of biological of terms used in this chapter.

Table 9.1. Glossary of biological terms used in Chapter 9

Blastocyst	An early stage of embryonic development. In the mouse it occurs between 3.5-4.5 days post fertilization.
Caudal	Towards the tail
Culture / cell culture	A medium that supports the growth of cells outside their normal environment. Used to nurture very early embryos during the production of GAAs
Dorsal	On or towards the back of an animal
Embryology	The study of the growth and development of embryos
Embryonic stem cells	Cells that develop around 3.5 days old (see blastocyst) that can give rise to any cell type in the body
Genome	All of an animal's genetic make-up
Genome editing components	A group of agents that enable the cutting of DNA and insertion of the required genetic material
Oocyte	Immature egg
Oocyte release	Ovulation, release of the oocyte from the ovary
Sterile male	A vasectomised male used to mate with females to stimulate pseudopregnancy
Stud male	A male that is kept for mating
Surrogate female	A female into which embryos are transplanted and that carries the pups to term and then rears them
Uterine tube	See Uterus. Older terms for uterine tube, which are still used, are fallopian tube and oviduct.
Uterus	In the mouse, the uterus us a Y-shaped organ. The V at the top of the Y is called the horns of the uterus and the l of the Y is the body of the uterus. The lower part of the body is the cervix (or neck). The horns lie close to the ovaries. Coiled tubes, called uterine tubes, are attached to each horn. At their free ends, the uterine tubes open out to a funnel shape called the infundibulum When ovulation occurs and oocytes are released from the ovary, they enter the infundibulum and start their journey down the uterine tube. The largest section of the uterine tube is the ampulla; it is here that fertilisation takes place
Ventral	The front of an animal (opposite direction of dorsal)

Source: Stephen Barnett

Genome-edited mice are generated by two main approaches. The first is to introduce genetic material and genome editing components directly into very early embryos, typically at the one-cell stage. The second approach involves genetically modifying mouse embryonic stem cells (mESCs) in culture first, and then microinjecting the stem cells into a later-stage embryo (typically 3.5 days old). Both techniques require the ability to access or generate embryos, maintain their viability in a cell culture environment and, after manipulation, transfer them into a surrogate female to generate a genetically modified mouse. Likewise, both techniques have been transformed by developments in the efficiency and ease of use of genome editing tools. The ability to alter the genetic code of mice has therefore required breakthroughs in embryology, stem cells and molecular genetics. The following paragraphs will describe the development of these techniques and how they have been brought together to allow for highly efficient and precise genome editing in the mouse. A short list of further reading is provided at the end of the chapter for those seeking a more detailed treatment of the topics covered.

Key developments in mouse embryology have allowed for the isolation and manipulation of embryos in a cell culture dish before returning them to a surrogate female. To increase the number of oocytes that are generated by each female, a technique called superovulation has been developed. This technique involves two injections of hormones, 48 hours apart, that first stimulate oocyte maturation, and then oocyte release. Using this technique, the number of oocytes released and then fertilised after mating can be increased manyfold, depending on the mouse strain. To obtain fertilised embryos,

female mice are paired with stud males on the day of the second injection for mating overnight. The next day the females are culled and the oviducts removed. Under a stereo microscope, and in a small drop of tissue culture medium, an area of the uterine tube called the ampulla is torn and the embryos, which are still at the one-cell stage, are released. The one-cell embryos, called zygotes, are usually surrounded by a large number of protective cells called cumulus cells. An enzyme, hyaluronidase, is then added to the tissue culture medium, which causes the cumulus cells to dissociate from the embryos. The zygotes can then be collected for genetic manipulation.

Three different methods have been developed for introducing genetic material and genome editing components into the zygote. The first of these involves microinjection using a fine glass needle attached to a micro-manipulator; a skilled operator and specialised equipment are needed to perform this. This method has been used successfully to introduce very large DNA molecules into embryos, which typically randomly integrate into the genome. More recently, a simpler method called electroporation, involving passing pulses of electric current through the embryos while in a solution containing the genome editing components, has been shown to be very effective for introducing small, targeted changes to the genome, such as single base-pair 'point mutations'. For larger targeted edits, including introducing the sequence for a fluorescent protein such as green fluorescent protein into a specific mouse gene to track its expression in live cells, the DNA and gene editing components are often delivered to the zygote packaged into a virus, in particular an adeno-associated virus (AAV). The use of AAV has been proven to be very efficient in delivering genome editing components into the zygote; however, AAV is still limited in the size of the DNA it can deliver, and invariably requires a higher level of biosecurity to ensure the safety of users.

Embryos surviving the manipulation process can either be immediately surgically transferred into surrogate females or be incubated overnight in tissue culture medium and transferred the next day at the two-cell stage. The latter has the advantage of identifying and discarding any non-viable embryos that have been damaged by the manipulation process. In order to act as a surrogate, a female mouse needs to have undergone mating with a sterile male to induce a state known as pseudopregnancy. The act of mating triggers hormonal changes in the female that are required to successfully carry a pregnancy. The embryos are implanted into anaesthetised (with perioperative analgesia) pseudopregnant females that have been prepared for surgery by shaving the dorsal caudal area and cleaning the skin using a suitable disinfectant, for example chlorhexidine. Under a stereo microscope, the microinjected embryos are loaded into a fine glass pipette with small air bubbles surrounding the embryos. The mouse is placed, ventral side down, under the stereo microscope and a small incision is made that exposes the reproductive tract. The embryos are then pipetted into the infundibulum. The presence of the air bubbles in the oviduct indicates a successful transfer. If needed, this can be repeated on the other side of the reproductive tract. The incision is then closed, and the mouse allowed to fully recover from anaesthesia in a heated chamber or on a heat pad. An important refinement that is increasingly being adopted is the development of a non-surgical method to transfer mouse embryos to a surrogate female. The non-surgical embryo transfer device is a pipette with a fine catheter that can be passed through the cervix to deliver the embryos directly into one of the uterine horns of a conscious pseudopregnant female. This method avoids the need for surgery and offers a significant improvement to the welfare of the recipient mouse, but is suitable only for later-stage embryos (after day 2.5 of development).

It is important to note that, despite the methods described above that target the one-cell embryo, editing can often occur after the zygote has divided, resulting in genetically mosaic offspring, with some cells having undergone genome editing and others not. Moreover, the genome editing reagents can create unwanted changes both at the targeted site and at other places in the genome. As a result,

it is invariably necessary to breed these mice and screen for offspring that have only the desired genetic alteration.

For larger or more complex targeted genome editing, an alternative approach to that of directly modifying the zygote is to genetically engineer mESCs in culture first and inject these into a recipient mouse embryo. mESCs are made by isolating cells of the inner cell mass of a later-stage pre-implantation embryo known as a blastocyst. Under the correct cell culture conditions, these cells can be expanded and maintained in an undifferentiated state indefinitely, undergo genetic modification and, when injected back into a recipient embryo, have the capacity to contribute to all the developing tissues of the mouse, including the germ line. One shortcoming of this method is that the mouse produced will often be derived from both the host cells of the embryo and the injected mESCs, to create what is known as a chimera. In such cases it is then necessary to extensively breed these chimeric mice to generate mice that are composed entirely of cells derived from the genetically modified mESCs. In some cases, large numbers of mice without the desired genotype may need to be culled.

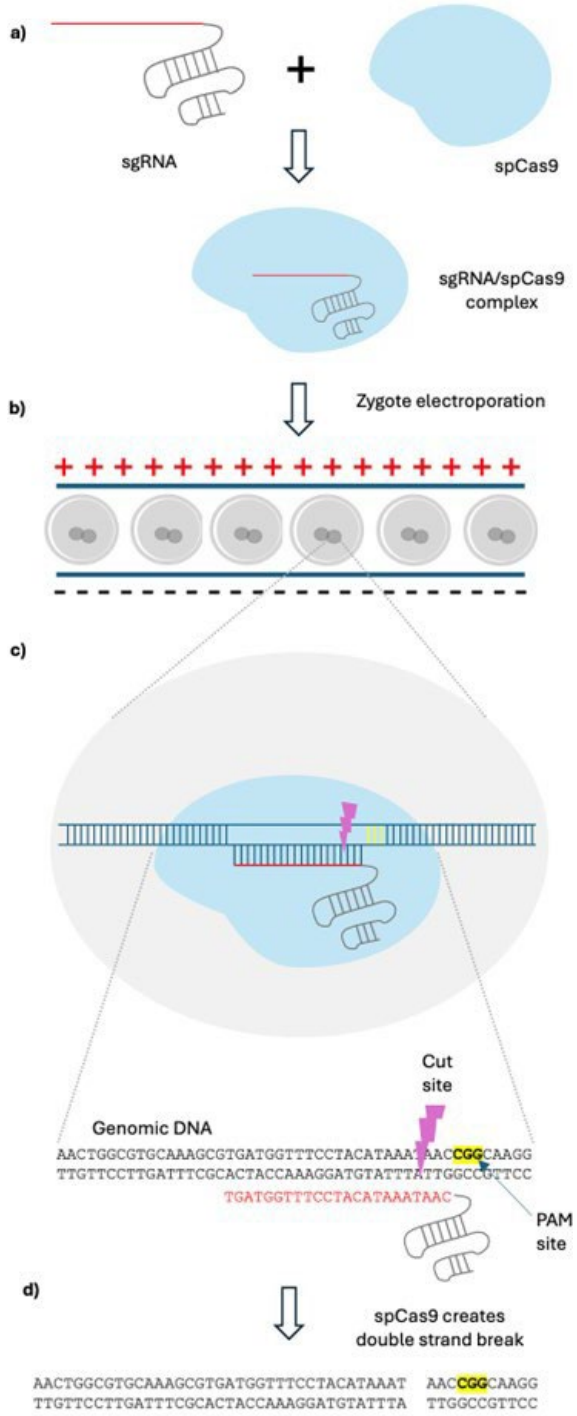
The development of efficient targeted genome editing required two important insights. The first came from the demonstration that a foreign DNA sequence could be introduced into specific sites in cells by flanking the foreign DNA sequence with the corresponding sequence in the genomic region that is to be targeted. When the foreign DNA is introduced into the cell by electroporation, the flanking sequence facilitates the introduction of the foreign DNA into the target site through a process called homologous recombination. However, the integration of the foreign DNA using this method is very inefficient. To enhance this process, genes that encode for antibiotic resistance can be included within the flanking sequences. This allows cells that have stably integrated the DNA sequence to be selected for, as these will survive when the antibiotic is added to the cell culture media, and those where the DNA has not integrated will die. Stem cell clones with the correct genetic modification are then grown so that they can be injected into embryos. This process of changing genes in stem cells has also been made much more efficient by the genome editing technologies explained below.

The second key insight for efficient targeted genome editing was that making a double-stranded break in the DNA at the location where an edit was desired greatly increased the efficiency of the integration of the foreign DNA. This realisation led to the development of a succession of increasingly efficient molecular tools for making targeted breaks in the genome to facilitate genome editing.

The technique known as clustered regularly interspaced short palindromic repeats (Crispr) was discovered as a bacterial system that binds to and destroys invading viruses. The name comes from the characteristic way in which the system is encoded in the bacterial genome. Research groups have adapted the Crispr system to operate as a simple programmable genome editing tool in mammalian cells. This work resulted in the award of the Nobel Prize in Chemistry in 2020 to Emmanuelle Charpentier and Jennifer A. Doudna. The system that was developed first, and that is most widely used, involves the Crispr-associated protein 9 (Cas9) from the bacterium Streptococcus pyogenes (SpCas9) acting as the endonuclease that cuts the target DNA. This system requires the identification of a 20-nucleotide (20-nt) target sequence preceding what is known as a protospacer adjacent motif (PAM) sequence, or PAM site, the presence of which is required for SpCas9 to cut. The PAM site for SpCas9 is the sequence 5′-NGG-3′, where N can be any nucleotide. The 20-nt target sequence can then be incorporated into a single guide RNA (sgRNA) molecule that is able to complex to the SpCas9 nuclease protein in a simple buffered solution (**Figure 9.1a**). After electroporation or microinjection of this sgRNA/SpCas9 complex into cells such as zygotes or mESCs, the complex will be transported to the nucleus as a result of mammalian nuclear localisation signals that have been incorporated into SpCas9 (**Figure 9.1b**). In the nucleus, the complex can specifically target the genomic site through

complementary base pairing between the sgRNA and the target sequence (**Figure 9.1c**). After target recognition, SpCas9 makes a double-stranded break in the genomic DNA (**Figure 9.1d**).

Figure 9.1. Schematic of Crispr/SpCas9 creating a double-stranded DNA break in zygotes.
(a) Complexing of an sgRNA with SpCas9.
(b) Electroporation of the sgRNA/SpCas9 complex into mouse zygotes.
(c) Complementary base pairing of the sgRNA with the genomic target DNA.
(d) SpCas9 cuts the target DNA 3–5 bp 5′ of the PAM site. (3-5 bp is the DNA target and PAM (protospacer-adjacent motif), is used to mark/identify the proper target site).



Source: Graham Cocks, KLC

The frequency of 'NGG' sites in the genome, combined with the ease of synthesis of the two components and the efficiency with which the Crispr/SpCas9 system can be delivered to cells, has made this a powerful and widely used genome editing tool in a wide range of organisms, including the mouse. Nevertheless, following on rapidly from the initial development of this Crispr/SpCas9 system, many research groups have adapted the system to target DNA with greater specificity and flexibility by altering how the system recognises target sites, including relaxing the requirements of the PAM site. This is a very active and productive area of research, and further innovations are expected that will increase the effectiveness of this already very powerful technology in the future. Whatever the future developments in the field of genome editing in the mouse may be, it is already clear that Crispr is a technology that is having, and will continue to have, a transformational impact on our fundamental understanding of mammalian biology and human disease.

9.4. Monitoring

Genetically altered mice have to be monitored extremely carefully so as to maintain the required genetic alteration within the colony and, individually, to check for any welfare issues related to the genetic alteration.

To maintain the required genetic alteration, the animals are bred in the same way as inbred mice (see **Chapter 8.6.1**), and regular genetic monitoring should also take place using genotyping techniques, for example polymerase chain reaction.

It is important that the colony is large enough to maintain the genetic alteration. If you rely solely on a few pairs of mice in the colony, the potential of genetic drift can be significant (see The Jackson Laboratory reference below). However, it is equally important not to have too large a colony, so that surplus animals are not bred. Therefore, good colony management is essential (NC3Rs, 2024).

The welfare of individual genetically altered mice should be checked carefully in the same way as for other mice (see **Section 4**). In addition, careful consideration should be given to any potential effects of the genetic alteration. For example, if the genetic alteration can give an increased incidence of mammary tumours in mice of a certain age, you should take particular care to examine the mammary area.

Accurate records of all observations should be kept. These will not only record the individual animals' welfare, but also show strain trends, including adverse effects, over time.

References

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, OJ L 276, 20.10.2010, p. 33, ELI: https://eur-lex.europa.eu/eli/dir/2010/63/oj/eng.

NC3Rs (2024), 'Colony management best practice', NC3Rs website, https://www.nc3rs.org.uk/3rs-resources/breeding-and-colony-management/colony-management-best-practice.

Further reading

European Commission, 'Animals in Science – Implementation', European Commission website, https://environment.ec.europa.eu/topics/chemicals/animals-science_en#implementation.

Larson, M. A. (ed.) (2020), Transgenic Mouse: Methods and protocols, Methods in Molecular Biology, Vol. 2066, Springer.

The Jackson Laboratory, 'Strain background and genetic drift', The Jackson Laboratory website, https://www.jax.org/jax-mice-and-services/customer-support/technical-support/strain-background-and-genetic-drift.

Wang, J. Y., Doudna, J. A. (2023), 'Crispr technology: A decade of genome editing is only the beginning', *Science*, Vol. 379, No 6629, eadd8643, https://doi.org/10.1126/science.add8643.

Contribution of this chapter to the Three Rs

The comments made in **Section 8** are relevant to this chapter. The potential for genetic editing to cause harm to animals in unexpected ways shows the importance of constant vigilance in monitoring their well-being and maintaining records of any problems reported.

Self-assessment questions

1. Give five examples of how GAAs can be used in science.
2. Monitoring the welfare of animals is always important. Why is it particularly important with GAAs?

10. Marking and identification of animals

After reading this chapter, you should be able to name different methods for marking and identifying individual animals and state the advantages and disadvantages of each method (learning outcome 4.8).

10.1. Introduction

Individual animals must be identified so that accurate records can be kept of their source and their experimental, breeding and health histories. In the case of genetically altered animals (GAAs), it allows a specific genotype to be linked to a specific animal (Dahlborn *et al.*, 2013).

Identifying animals is probably the most common technique carried out on laboratory animals. It is accepted that most methods cause transitory pain and discomfort, but there is evidence that little time is spent considering ways to reduce the discomfort (Klabukov *et al.*, 2023). Dahlborn *et al.* (2013) make the point that even a minimal reduction in discomfort would have a large overall refinement effect because of the very large number of animals involved.

It follows that careful consideration should be given to choice of method of identification used in each situation. When more than one method would be suitable, the one causing the least discomfort to the animal should be chosen. In all cases the method chosen must be acceptable to the scientist conducting the study.

What follows is not intended to be an instruction manual on how to carry out the identification process; the purpose is to outline some of the commonly used methods. The application of identification marks should be carried out only by an individual deemed competent after being instructed by a skilled colleague.

This chapter will describe the advantages and disadvantages of commonly used methods of identification.

10.2. Legal considerations

Article 32 of Directive 2010/63/EU (the Directive) requires that each dog, cat and non-human primate is provided with a permanent, unique identification mark. The mark must be applied by weaning age in the least painful manner possible. The identification of other species is not mentioned in the Directive but Member States may implement their own criteria (Dahlborn *et al.*, 2013).

10.3. Selecting the most suitable method of identification

When selecting a suitable method, the following factors should be considered.

— Effect on the animal: the method should cause the minimum pain and distress possible when applied. Analgesia should be considered. There should be no subsequent discomfort through irritation, constriction, infection or toxic effect.

— Application and maintenance: the method should be simple to apply with the minimum amount of restraint.

— Clarity of the mark: the identification mark must be easy to read, preferably without handling the animal. If the identification is in code form, it should be simple to decipher (that means the code must be easily understood).

— Length of time the mark needs to last: in many cases, the mark must last for the whole life of the animal; in others, the mark need last only a short time.

— Possible interference with experimental needs and other activities: for example, an electronic tag would not be suitable for some cancer research studies (see **Chapter 10.4.2 Microchip Transponder**).

10.4. Commonly used methods of identifying laboratory animals

Identification methods in this chapter are divided into non-invasive (e.g. physical characteristics, applying stains) and invasive methods (e.g. ear tags, microchips). As the name suggests, invasive methods may cause some pain and distress. Non-invasive methods do not cause pain, but, as they can require restraint, may cause some distress. Habituation and positive reinforcement training greatly reduce the distress caused by restraint, and therefore help to refine the procedure (see **Section 13.2**). Whenever possible, non-invasive methods should be favoured over invasive ones.

Rapid application by skilled care staff will reduce the discomfort, as will the application of an analgesic when appropriate. As with all procedures carried out on laboratory animals, aftercare is important. It is possible that a poorly applied mark could cause infection, irritation and discomfort. Post-application inspection will identify these problems so they can be rectified.

10.4.1. Non-invasive methods

Describing the physical characteristics of animals

Small numbers of animals that have distinctive coat colour distribution can be identified by photographing the whole animal. **Figure 10.1** shows how a group of rabbits with distinctive colours can be kept together and be identified.

The main advantage of this method is that:

— it causes no pain and usually no distress.

The disadvantages of this method are that:

— it can be used only for very small numbers of animals;

— it can take some time to identify specific animals;

— few strains have easy-to-distinguish coat variations.

Figure 10.1. Rabbits with variation in coat colour housed together

Source: Sarah Lane

Shaving or cutting fur

Removing fur from the backs of animals is a painless method of identifying them. Although painless, it will cause some distress because the animal will need to be restrained.

Hair is removed with scissors or hair clippers. A code can be devised whereby different areas of the back are allocated different numbers, but, even with a code, only small numbers of animals can be identified in this way. The method will need to be repeated when the hair regrows.

The advantages of this method are that:

— the method is painless;

— the equipment is simple to use. The disadvantages are that:

— only small numbers can be identified;

— the method is temporary;

— if a mistake is made, it cannot be rectified until the hair regrows.

Applying stains

Coloured markers can be used to stain the fur or tail skin of light-coloured animals. The method is suitable for temporary identification only, because it will wear off quickly. If longer-term identification is required, the marking has to be repeated at least every 10 days (**Figure 10.2**).

Marking can be done with food colouring, human hair colouring agents or felt- or fibre-tipped pens containing non-water-soluble ink. The solvent for the ink must be checked to ensure that it will not harm the animals (particularly very young ones) or affect experimental results. Stains are applied with cotton buds or with a fine paintbrush.

Stains should be applied in small patches and be worked well into the fur. It is important to ensure that stains are dry before replacing animals in their groups, as the stains may transfer to other animals.

Codes for this method are easy to learn only for fairly small numbers of animals. The code must be displayed where the animals are housed or used. Staff with colour blindness may have difficulty interpreting the marks.

The advantage of this method is that:

— it is painless and easy to apply.

The disadvantages of this method are that:

— it is temporary;

— only a small number of animals can be identified;

— the animals need to be restrained during application and drying;

— care must be taken to ensure that the dyes used do not harm the animals or affect experimental results.

Figure 10.2. Identification stains on guinea pigs

Source: Stephen Barnett

Leg rings

The use of leg rings is restricted to birds (**Figure 10.3**). In the past they were used for rabbits, but that method is no longer recommended. Leg rings are available in two forms: closed rings consisting of a continuous band of material, and split (or adjustable) rings. Rings can be colour-coded and/or bear numbers or text. The method is non-invasive, but the animal has to be handled for the ring to be read.

Closed rings for birds are species specific. The correct-sized ring must be slipped over the foot of the young bird (normally at approximately seven days of age). The foot of the young bird is lubricated, the three forward toes are placed into the ring and it is slipped over the ball of the foot and up the leg until the hind toe is free of the ring. Once the leg and foot reach their full size, the closed ring will not slide off over the foot. Split rings may be applied to the bird at any stage in its life, because they stretch as the bird grows.

The advantages of this method are that:

— it is painless to apply;

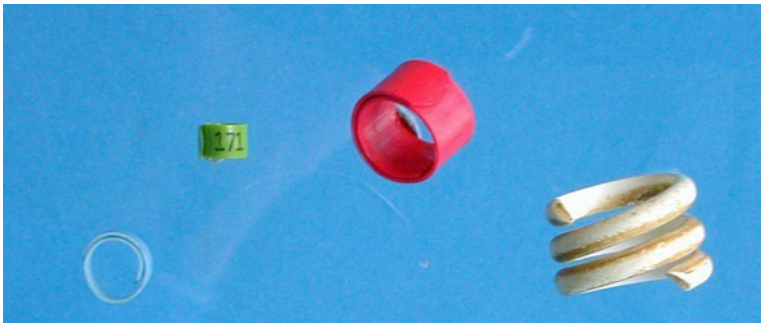
— large numbers can be identified.

The disadvantages of this method are that:

— the animal must be handled to apply and read the ring;

— closed rings must be checked to ensure that they do not become too tight.

Figure 10.3. Chicken leg rings



Source: Stephen Barnett

10.4.2. Invasive methods

Invasive techniques break the skin, so all equipment in contact with exposed tissue must be sterile.

Ear punching or notching

This technique involves removing small pieces of the pinnae of the ear of a rat or mouse in a coded pattern (**Figure 10.4**). The hole is made using a special punch. Although the word 'punch' is used, the instrument should 'cut' a hole, and it should be sharp. Alternatively, surgical iris scissors (short, straight and sharp scissors used for cutting fine structures) can be used to cut a V or notch around the edge of the ear. The code used should remove the minimum number of ear pieces. Codes should be simple, as complicated codes are difficult to read and cause unnecessary discomfort to the animals.

Ear punching and notching does cause pain and should be replaced by other methods unless it can be justified (Burn *et al.*, 2021). The fragments of ear removed can be used for genetic analysis; if it is intended to do this, the punch/scissors must be completely cleaned between animals to prevent genetic cross-contamination.

The main advantages of this method are that:

— it is cheap to apply;

— the tissue removed can be used for genetic analysis.

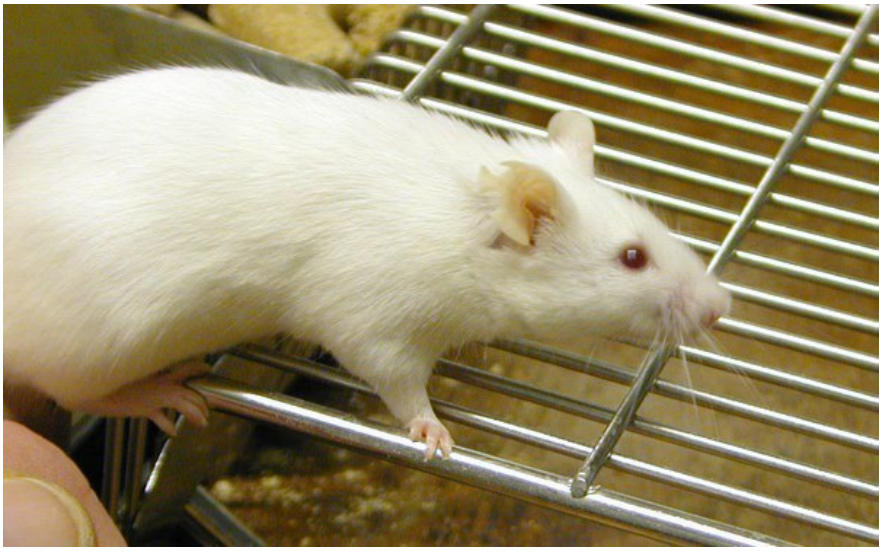
The disadvantages are that:

— it causes pain;

— the animals have to be restrained to read the code;

— ears may be damaged if animals fight.

Figure 10.4. Ear punch



Source: Stephen Barnett

Microchip transponder

This method consists of two main components, a transponder chip and a reader. The chip is small (from 6 mm long by 1.4 mm wide) and either is preprogrammed with a unique alphanumeric code or can be programmed by the user. It is injected subcutaneously (under the skin) in the neck region of the animal. When a reader is passed near the animal, it sends out an electromagnetic pulse that triggers the transponder to send the animal's identification number. The number is then displayed on the reader's screen (**Figure 10.5**). Information can be stored in the reader and downloaded onto a computer at a later stage.

The transponder is supplied with an applicator fitted with a needle to make the injection (**Figure 10.6**). The needle is larger than would be considered suitable for small animals, so inhalation anaesthesia should be used when implanting transponders in rodents. After insertion, the wound should be surgically closed (Dahlborn *et al.*, 2013).

The main advantages of this method are that:

— it is permanent;

— very large numbers of animals can be identified;

— the readers can be connected to scales and other equipment so that measurements can be directly linked with an animal's identity.

The main disadvantages are that:

— the application is painful;

— the application is time-consuming;

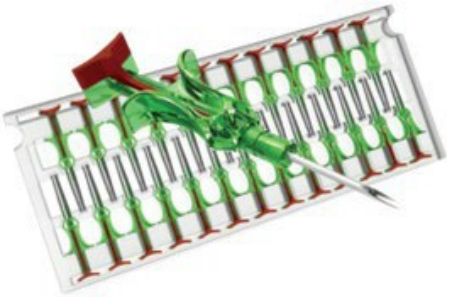
— the transponder has been associated with inflammation and tumour growth over time;

— the equipment is expensive.

Figure 10.5. Transponder reader

Source: Plexx BV

Figure 10.6. Transponders and applicator



Source: Plexx BV

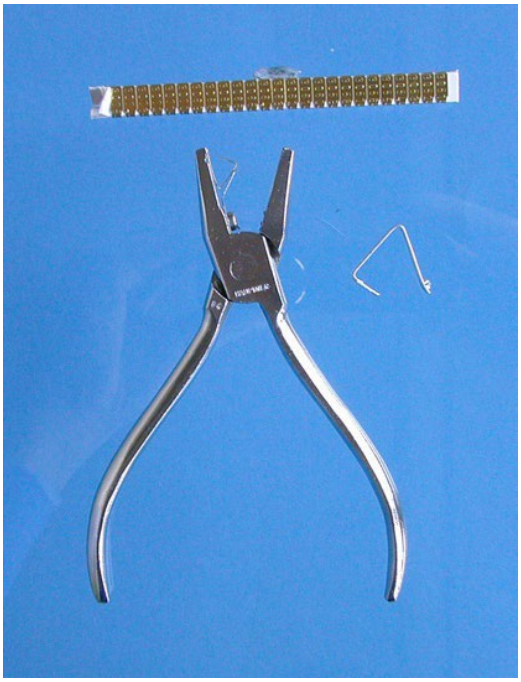
Ear tags and studs

A variety of studs and tags are available to fit most species of animals, from small rodents to large farm animals. They are made from metal or plastic. Some are self-piercing (**Figure 10.7**); others require a hole to be punched first, so they cause pain when first applied. They can be colour-coded in addition to being numbered, which allows for the identification of both groups and individuals.

Two main types of tag are available, Ketchum™ tags and Rototags™. Ketchum tags (**Figure 10.7**) enclose part of the outer edge of the ear and must be placed with care or the ear may grow around them. Alternatively, if too much room is allowed for growth, the animal may catch the tag and pull it out. Rototags and studs (**Figure 10.8** and **Figure 10.9**) do not have this problem, as they do not enclose the ear. Tags should be positioned close to the bottom of the ear so the weight of the tag does not pull the ear down.

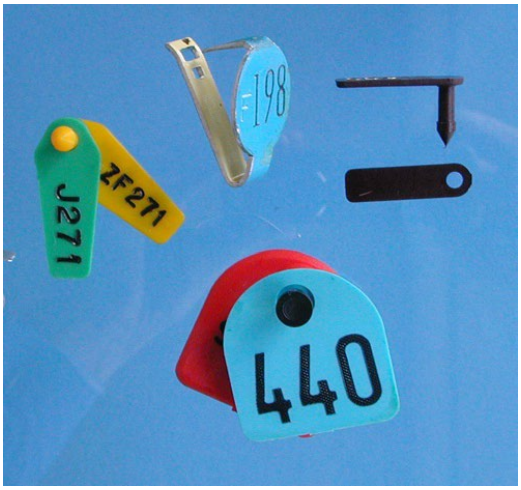
Lightweight plastic mini-tags are available for rodents. The tags are etched with a barcode, which can be read with a normal barcode reader.

Figure 10.7. Ketcham tags with pliers



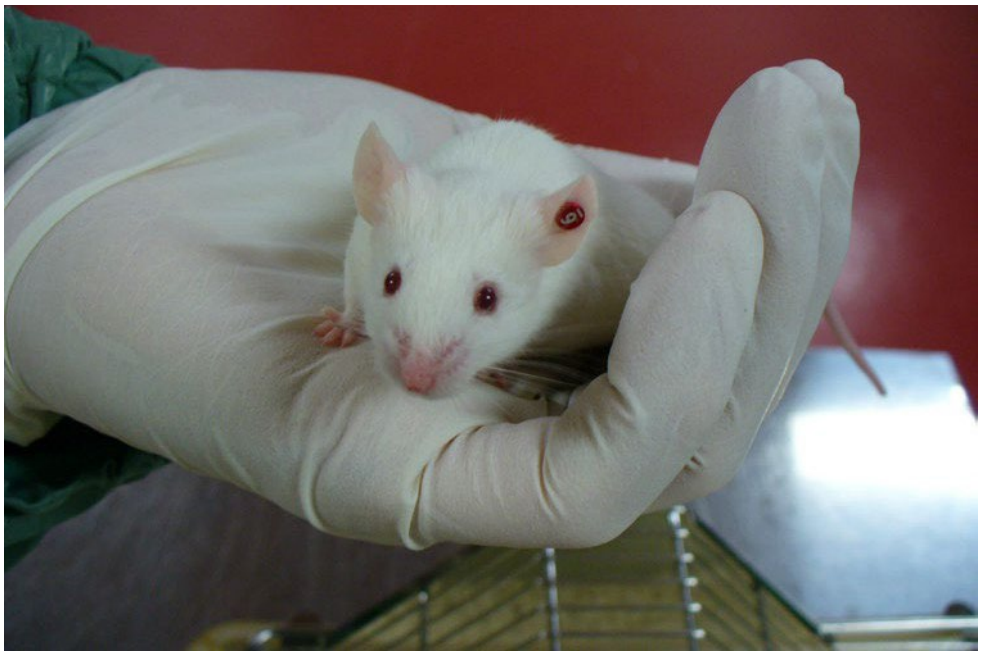
Source: Stephen Barnett

Figure 10.8. Assorted Rototags and one Ketchum tag



Source: Stephen Barnett

Figure 10.9. Young rats fitted with ear studs



Source: VetTech Solutions / Stoelting

The advantage of this method is that:

— large numbers of animals can be identified.

The disadvantages of this method are that:

— it causes pain when applied;

— regular checks must be made to ensure that the tag is not damaging the ear;

— small animals must be handled to read the tag;

— tags occasionally get caught in caging and are torn out.

Tattooing

Tattooing is a technique that introduces dyes into the dermal layer of the skin. If applied properly, it is permanent, although the ink may leach into surrounding tissue, making the tattoo indistinct over time. The tattoo can be applied to the ear or, in small animals, the tail or toes (**Figure 10.10**).

The main method of applying the tattoo is by using a tattoo pen (**Figure 10.11**). An electric tattoo pen has a vibrating needle that can be used to write the identification code freehand.

Skill is needed to apply the mark while causing the minimum pain and distress to the animal. Tattoo ink is available in white, green and black, so dark-skinned animals can be tattooed.

Micro-tattooing devices are available to mark the ears, tails and toes of rodents. Simple codes can be used, for example a dot on one toe and a dot on a different toe of another animal in the same cage.

Figure 10.10. Ear tattoo on a rabbit

Source: Stephen Barnett

Figure 10.11. Tattoo pen

Source: VetTech Solutions

The advantages of this method are that:

— large numbers of animals can be identified;

— it is long term, especially in rodents;

— it is easy to read;

— it can be applied to neonatal mice and rats. The disadvantages of this method are that:

— it causes pain when applied;

— small animals need to be restrained to read the mark;

— applying the mark is time-consuming;

— the mark fades over time;

— in animals with hairy ears, the ears have to be shaved in order to read the mark.

References

Burn, C. C., Mazlan, N. H. B., Chancellor, N. and Wells, D. J. (2021), 'The pen is milder than the blade: Identification marking mice using ink on the tail appears more humane than ear-punching even with local anaesthetic', *Animals*, Vol. 11, No 6, p. 1664, http://doi.org/10.3390/ani11061664.

Dahlborn, K., Bugnon, P., Nevalainen, T., Raspa, M., Verbost, P., *et al.* (2013), 'Report of the Federation of European Laboratory Animal Science Associations Working Group on animal identification', *Laboratory Animals*, Vol. 47, No 1, pp. 2–11, https://doi.org/10.1177/002367712473290.

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, OJ L 276, 20.10.2010, p. 33, ELI: https://eur-lex.europa.eu/eli/dir/2010/63/oj/eng.

Klabukov, I., Shestakova, V., Krasilnikova, O., Smirnova, A., Abramova, O., *et al.* (2023), 'Refinement of animal experiments: Replacing traumatic methods of laboratory animal marking with non-invasive alternatives', *Animals*, Vol. 13, No 22, p. 3452, https://doi.org/10.3390/ani13223452.

Contribution of this chapter to the Three Rs

This chapter emphasises the need to select the least painful and distressing method of marking animals in any situation and the need to consider the use of analgesia when necessary. It mentions the importance of habituation and training before animals are restrained for marking and how distress of animals is lessened by the application being carried out by skilled staff. All of these measures help to refine the procedures.

Self-assessment questions

1. What is the difference between invasive and non-invasive methods of marking animals?
2. Suggest three ways in which the distress an animal may experience during the application of identification can be minimised.
3. Describe an advantage and a disadvantage of using coloured markers for identification.
4. What are the disadvantages of using ear tags for rats?
5. What is the advantage of using microchip systems for identifying laboratory animals?

11. Minimally invasive procedures without anaesthesia and the welfare concerns associated with them

After reading this chapter, you should be aware of the theoretical principles of performing minor procedures on laboratory animals without anaesthesia (learning outcome, module 7). You should also understand how stressful activities such as transport and minor procedures can be refined (learning outcome 4.12).

11.1. Introduction

Minimally invasive procedures include the administration of substances and the withdrawal of body fluids and tissues from animals. These procedures may cause discomfort and stress to the animals, so every opportunity must be taken refine them. The refinement should apply not only to the procedure itself, but also to what happens to the animals before and after the procedure.

Invasive procedures are performed without anaesthesia if the harmful effects of the anaesthesia outweigh the harmful effects of the procedure itself.

The information that follows is not intended to be a practical instruction manual. Procedures should out be performed only by staff who have undergone training with an experienced, authorised colleague and have been deemed competent.

11.2. Preparatory refinement

11.2.1. Transport

Minor procedures are carried out in procedure rooms, and animals have to be transported to these rooms. Animals find all transport stressful, even if it is a relatively short distance within a facility. The longer the distance travelled, the more stressed animals become; transportation by road or air is the most concerning.

The stressful experience of transportation can be mitigated by careful planning. When moving animals from one room to another in the same facility, it is necessary to confirm that the receiving room is ready to accept them before the journey is started so that they can be transferred into their new accommodation without delay. A route must be chosen that can be passed through without the risk of being held up and without any areas containing noisy equipment. The trolley or other method of transport must run smoothly so the animals do not get shaken, which would upset them.

When moving animals from external breeding establishments or from another laboratory, follow the same principles. All transportation of vertebrate animals in the EU must be done in accordance with Council Regulation (EC) 1/2005, which states that 'No person shall transport animals or cause animals to be transported in a way likely to cause injury or undue suffering to them.'

Council Regulation (EC) 1/2005 covers the following.

— A route and journey plan must be prepared before the journey commences, together with an estimate of the duration of the journey, and both sender and recipient must agree departure and arrival times.

— Containers must be suitable for the species being carried. Small laboratory animals must be transported in sealed boxes with filter material covering air inlets and a window in the top so the animals can be inspected on the journey (**Figure 11.1**).

— Animals must be declared fit to travel by a competent person. Sick animals should not travel unless they are under veterinary supervision or they are travelling to obtain veterinary assistance.

— Vehicles must be fit for purpose and well maintained. Farm animals must be easily loaded and unloaded and must be able to be inspected en route. Small laboratory animals must be transported in air-conditioned vans with a double-door system so that any animals that escape their boxes cannot escape from the van.

— Drivers and other attendants must be competent and knowledgeable about the animals they are transporting.

— Food and water must be provided on the journey. Food and a source of moisture can be provided in the containers of small laboratory animals. For farm animals, stops for rest, food and water should be incorporated in the journey plan.

It should be noted that, at the time of writing this manual (March 2025), the legislation is being revised. Readers are advised to check the legislation in force. Further details on the protection of animals during transport can be found at https://eur-lex.europa.eu/.

Figure 11.1. Transport box for small laboratory animals showing viewing window

Source: Stephen Barnett

11.2.2. On arrival

On arrival, the animals should be unpacked, inspected and placed in their new housing as quickly as possible. Initially, they should be placed in quarantine accommodation (see **Chapter 4.3.5**), to check that they are not carrying any disease causing agents.

Acclimatisation

The stress that animals experience when transported is both a welfare problem and a scientific one. The effect on their physiology will affect experimental results. They must be given time to recover from their journey and to get used to their new environment before any experimental work is started. This is necessary even if the movement is from a room close by to the procedure room. The noises, smells, staff, etc. will all be different, and the animals must be given time to get used to them. This settling-in time is called the acclimatisation period. The period that will be needed depends on the length of the journey, the species/strain and what biological system is being studied (Obernier and Baldwin, 2006).

Chapter 13 'Handling and sexing' describes how positive reinforcement training, habituation and socialisation are used to lessen the distress caused by handling and restraint. There is strong evidence that, if animals are trained to know what to expect when procedures are carried out, they are much less disturbed by them.

Appropriate transport, rapid housing on arrival and a suitable acclimatisation period, followed by the use of habituation and positive reinforcement training, all minimise the adverse effects on the animals. Well-trained, competent staff, who can carry out the techniques rapidly and carefully, add to the refinement of the procedure.

11.3. Minimally invasive procedures: techniques

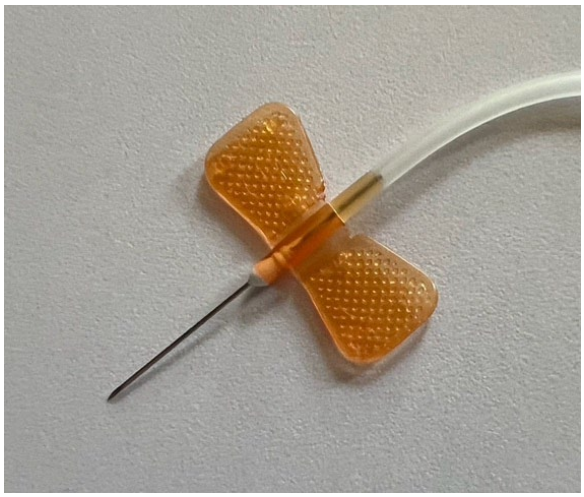
11.3.1. Administration of experimental substances

Test substances are administered to experimental animals in similar ways to those used to administer medicines in veterinary clinics. Several routes can be used to administer test substances. The one selected in any situation will depend on the species of animal, the nature of the substance being administered and the experimental requirement. The route that the principal scientist has decided should be used in any experiment will be stated in the experimental protocol.

Many of the routes discussed here require using hypodermic needles. Hypodermic needles come in assorted sizes and are of two main types. One is the type used to give vaccinations to or take blood from a human; it consists of a metal needle attached to a hub that fits onto the base of a syringe. The other type of needle is called a butterfly needle. In this case, the needle is attached directly to a flexible tube; where the needle joins the tube, thin plastic is attached, so it looks like wings on either side of the needle. The opposite end of the tube is fitted with a hub that connects to a syringe.

Butterfly needles are easier to insert, particularly in smaller veins (**Figure 11.2**). The flexible tube between the syringe and needle makes it less likely that the needle will pull out if the animal moves.

Figure 11.2. Butterfly needle



Source: Stephen Barnett

Needles are described by their gauge number (G) and their length, in millimetres. The gauge number is a shortened version of the Standard Wire Gauge and refers to the bore of the needle. The higher the G number, the smaller the bore. So a 25-G needle has a very narrow bore and a 16-G needle has a relatively wide bore. As a rule, the smaller the bore that can be used, the better; however, in deciding on the size of the bore, several things have to be taken into account.

— The route being used to inject the test substance (see **Chapter 11.3.2 Parenteral routes**).

— The nature of the material being injected. Water-soluble substances flow easily, but thicker liquids, such as fat- or oil-based ones, may block narrow needles, so a larger-bore needle must be used.

— The species and the thickness of the skin. Rodent skin is relatively thin and smaller needles pass through them easily. At the other end of the scale, an adult cow has very tough skin; a small-bore needle may not penetrate it and, if it does, the sharp point may burr (which is when the point of the needle bends over, creating a hook), causing more damage to the tissues and therefore more discomfort when it is withdrawn.

New sterile needles must be used for each animal to prevent cross-contamination and to ensure that the needle has a sharp point, as the point becomes blunt after one use. The substance to be injected will cause less pain if it is at the animal's body temperature.

The maximum recommended volumes that can be injected by each route are given in **Table 11.1**.

11.3.2. Routes of administration

Enteral routes

Enteral routes are those whereby substances are administered directly into the alimentary canal, where they can be absorbed into the circulatory system through the epithelial cells that line the wall of the intestines. Most enteral routes involve access through the mouth (also referred to as per os), but substances can also be administered enterally via the rectum. It is not a common route, but is appropriate in some cases.

Three main methods of enteral administration are used.

— Dietary.

- Inclusion of a test substance in food or water. If a test substance is added to food, it must be thoroughly mixed in so that the animal cannot avoid consuming it. In the case of small mammals that are fed pellets, the pellets are ground up, making it easier to mix in the test substance. Any addition to food or water must not affect its palatability (taste) or the animals will not eat it.

— In a capsule or tablet. These are used when an exact dose of a substance must be administered.

- Capsules are made of gelatine and supplied in two halves that can be filled and fitted together. For larger animals, such as dogs, the animal's mouth is opened wide, the capsule is placed as far back on the tongue as possible, the mouth is closed and swallowing is induced by gently stroking the throat. The dog should be observed because it may avoid swallowing and spit out the capsule later. Capsules can be used for test substances that may be unpleasant to take in the mouth voluntarily.

- Tablets. Test substances can be formulated into solid tablets similar to those taken to treat human diseases. They are administered as described for capsules.

— By gavage.

- Gavage means 'forced feeding'. It refers to techniques whereby test substances are administered directly into the stomach. A flexible tube connected to a syringe is used. The tube size should be approximately 16 G for adult rats. In the case of small animals, such as rats and mice, the animal is held by the scruff of the neck and body. The animal should be held tightly enough so that the head cannot move, but not so tight that the animal gets too distressed. Training the animal to be held in that way before the dosing has to take place should lessen the animal's stress. The gavage tube is held up against the animal to assess how far it has to go in to reach the stomach; then it is gently passed into the mouth and over the back of the tongue, which will elicit a swallowing reflex in the animal, and the tube will be directed into the oesophagus.

  If there is any resistance at all, the tube must be withdrawn, as it may have entered the trachea by mistake. A syringe is attached to the tube and the test substance slowly injected into the stomach. The optimum volume that can be administered by this method is 5 ml/kg of body weight (Turner *et al.*, 2011). Once the injection is complete, the tube should be slowly withdrawn, and the animal should be inspected to ensure that there are no immediate ill effects before it is returned to its cage. It should be kept under observation for a few days.

- Gavage can be used for large animals but they must be in a standing or sitting position and restrained by a second person.

Parenteral routes

Parenteral routes are all routes other than the enteral ones. They are administered by topical application or by introduction into the body by other means, usually by injection.

Topical routes. Applying substances directly onto the surface of the region of the body that is to be affected by them.

— Application of liquids, creams or pastes to the skin.

    - The most common site for topical application is the side of the body. Hair has to be clipped, taking care not to cause skin abrasion. Once the test substance has been applied, it should be covered so that the animal cannot lick it or rub it off.

— Drops into the nose, ear or eye.

— Inhalation of gases, vapours or aerosols for absorption via the lungs.

    - Special equipment is required for this type of administration, for instance an inhalation chamber such as the one used to administer anaesthesia. Animals must be acclimatised to the inhalation equipment before any administration takes place. Care must be taken to ensure that other animals and staff are not exposed to the test substances while they are being administered to the animals.

Other parenteral routes. All of the following are administered with a syringe and a hypodermic needle. Injection volumes by different routes are given in **Table 11.1**.

— Intradermal.

    - Injection of substances into the layers of the skin. The flanks and ears are the sites used most often. Hair is removed to give a clear view of the injection site. The needle (27 G is recommended for rodents) is passed through the dermal layer of the skin for about 5–10 mm and the injection made very slowly (as the needle is withdrawn). A small swelling will be seen (called a bleb). There will be a resistance to the injection, but it is important not to increase the pressure on the syringe. The bleb should not be rubbed. A maximum of 0.05 ml can be injected in a rodent.

— Subcutaneous.

    - Injection of substances into the layer of connective tissue immediately under the skin. The loose skin at the neck and the side of the animal are the most common sites.

— Intramuscular.

    - Injection of substances into a muscle. The large muscles in the thigh are often used, but even these are very small in rodents; therefore, only very small amounts should be injected (maximum 0.05 ml in mice). No more than two injections should be given in one day (one injection per site). Care must also be taken to avoid blood vessels, nerves and bone.

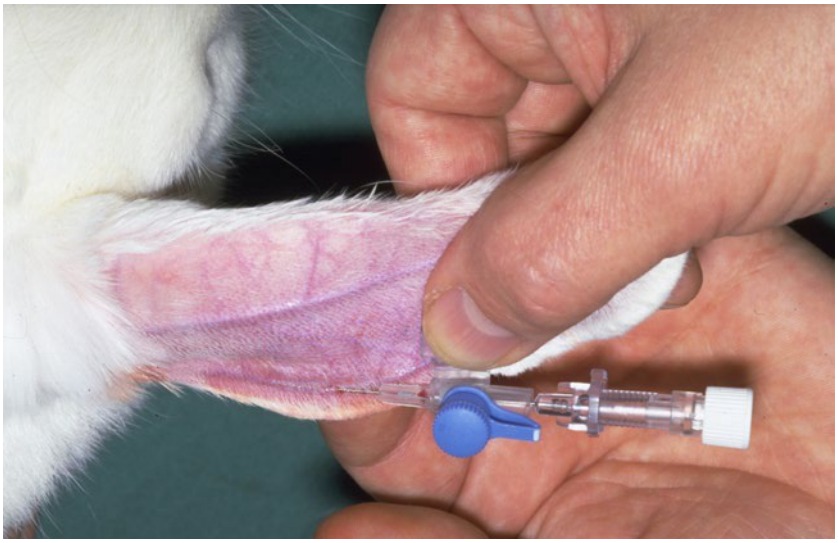
— Intra-peritoneal.

    - Injection of substances into the peritoneal cavity (the space between the abdominal wall and the abdominal organs). The needle must be positioned carefully so as to miss any internal organs. The needle is inserted in the lower left or right quadrant of the abdomen, away from the midline, so the bladder is missed. Only the tip of the needle should be inserted and the syringe piston should be withdrawn slightly to check that it has not entered any blood vessel or abdominal organ.

— Intravenous.

- Injection of substances directly into a superficial vein. In mice and rats, the tail vein is the most accessible; in rabbits and pigs, it is the ear vein (**Figure 11.3**); in dogs and cats, it is the cephalic vein, which runs down the centre of the foreleg; and in larger animals, such as sheep and cattle, the jugular vein is used. There are four stages to the injection technique: visualisation – make sure the vein can be clearly seen; stabilisation – prevent the vein from moving when the needle is inserted; occlusion – put pressure on the heart side of the site where the needle is to be inserted to 'raise the vein'; and haemostasis – put pressure over the exit wound when the needle is withdrawn to stop bleeding.

Figure 11.3. Inserting a needle into a rabbit's ear vein



Source: Newcastle University

The tail vein in mice and rats is easier to see if either the whole animal is warmed in a heated chamber at 28–30°C or the tail is warmed in water at 30–35°C. With other animals, the vein can be occluded on the heart side of where the needle is going to be inserted. Occluding the vein temporarily stops the blood getting back to the heart and causes the vein to swell, thus making it easier to see. It is important to remove the occlusion before the injection is started, or blood and the injection fluid will be forced out of the hole made by the needle, causing bruising and pain. After the injection is completed and the needle is carefully withdrawn, pressure should be applied with a sterile swab over the exit wound to stimulate clotting (haemostasis).

Table 11.1. Maximum injection volumes (ml/kg of body weight)

Animal	Subcutaneous	Intra-peritoneal	Intramuscular (ml/site)	Oral	Intravenous
Mouse	10	20	0.05	10	5
Rat	5	10	0.1	10	5
Guinea pig	5	5	0.1	10	5
Rabbit	1	5	0.25	10	2
Cat	1	1	0.25	5	2
Dog	1	1	0.25	5	2.5

NB: These figures relate to healthy, adult animals

Source: This table has been adapted from Waynforth (2007).

Demonstrations of these techniques can be found on the 'Procedures with care' web page of the NC3Rs resource library (https://nc3rs.org.uk/3rs-resources/procedures-care).

11.4. Collection of samples

Tissue, blood, urine and faecal samples are routinely collected for analysis as part of scientific projects or for health reasons.

11.4.1. Tissue biopsy

Tissue biopsy involves taking samples of tissue or cells from an animal so they can be analysed in a laboratory. Taking tissue samples will usually require anaesthesia, and those techniques are outside the scope of this chapter. However, care for the animal both before and after the procedure should follow the same pattern as for the procedures mentioned in this chapter. Sometimes tissues removed for other reasons can provide tissues for analysis; for example, ear sections that are removed to identify animals can be used for genotyping (genetic screening). Other examples of cells used for diagnostic purposes are blood for differential blood cell counts, and vaginal cells to identify which stage of the oestrous cycle an animal is in, see Vaginal smear (see **Chapter 8.2**).

11.4.2. Blood

Many scientific studies require blood to be taken from an animal either periodically or as single sample. Blood samples are most often collected using the intra-venous route, although, for some experimental purposes, arterial blood may be used. The amount of blood that may be safely taken from an animal depends on the total blood volume of the animal and the frequency of sampling. The total blood volumes (TBV) of selected species are given in **Table 11.2**.

When removing blood, less than 10% of TBV should be removed at any one time and less than 15% should be removed in any 28 day period. (See NC3Rs 2022, Resource library, Blood Sampling for more information).

Advances in biochemical analysis have made it possible to work with samples of less than 50µl. Where micro-sampling, like this, can be used it minimises the adverse effects of blood sampling. If regular samples are required indwelling catheters can be used to avoid exposing animals to repeated puncture wounds and tissue damage.

Table 11.2. Total blood volumes

Animal	TBV (ml/kg)
Mouse	58.5
Rat	54–70
Guinea pig	69–75
Rabbit	57–65
Cat	47–65
Dog	70–110

NB: These figures relate to healthy, adult animals

Source: Adapted from Wolfensohn and Lloyd (2003).

Blood-collecting techniques

Venepuncture

Collecting blood by nicking a superficial blood vessel is called venepuncture. When only a drop or two of blood is required from a mouse, the animal may be placed in a restrainer with its tail protruding. The tail is cleaned with a skin antiseptic and the area is dried. A small puncture is made in the tail

vein with a sterile needle. The drop of blood that forms can be transferred directly onto a slide for smearing or can be collected in a capillary tube. When the blood has been collected, pressure is applied with a sterile swab to the wound to encourage clotting. If the technique is applied correctly, the animal will suffer minimum discomfort.

An alternative to the tail vein is the saphenous vein. This runs down the outside (lateral aspect) of the hind legs. The animal is restrained, and the leg is shaved, cleaned with skin antiseptic and dried; the vein is punctured and blood collected as described above. Blood collected by venepuncture will not be sterile.

Aspiration from a vein

If larger samples of sterile blood are required, they are best obtained directly from the circulatory system by means of a hypodermic needle.

An adult rabbit may have several cubic centimetres of blood removed from an ear vein using the following technique. The rabbit is restrained by an assistant or placed into a restraining box. The ear is shaved with clippers to make the vein more visible, and is disinfected to prevent infection. The vein may be dilated (raised) by warming or gently massaging the ear and occluding the vein close to the head with the finger and thumb. This occlusion must be removed once the needle is in the vein.

A syringe fitted with a suitably sized sterile hypodermic or butterfly needle is used to puncture the skin and vein and is inserted until the needle lies comfortably within the vein. As the vein is entered, blood may flow into the hub of the needle under venous pressure. The syringe piston is slowly withdrawn to allow blood to flow into the barrel of syringe. If blood is withdrawn too quickly, the vein will collapse, stopping the flow altogether, damaging the vein and damaging the blood cells. After the needle is withdrawn, a sterile swab should be pressed over the puncture wound to encourage the blood to clot.

If blood is to be collected from a rat or mouse, the lateral tail vein is used. In larger animals such as dogs, the cephalic vein in the foreleg is a common site.

Demonstrations of these techniques and blood collection from other laboratory animals can be found on the 'Blood sampling' web pages of the NC3Rs resource library (https://nc3rs.org.uk/3rs-resources/blood-sampling).

Vacuum collecting systems

Vacuum systems consist of a double-ended needle that is attached to a plastic syringe barrel, but the normal syringe piston is replaced by a tube with a rubber stopper (**Figure 11.4**). There is a vacuum in the tube. The external part of the needle is used to puncture the vein and, when blood enters the barrel, the glass tube is pushed down onto the internal end of the needle so that the stopper is punctured. This causes the blood to be drawn into the tube. The inner surface of the tube can be coated with anticoagulants if the study requires them. This system is suitable only for bigger veins in larger species.

Figure 11.4. Vacuum system for blood collection, showing exterior needle, barrel and vacuum tube

Source: Gareth Gough, Bristol University

11.4.3. Collecting samples of faeces and urine

The technique used to collect faecal and urine samples varies depending on the amount needed and what they are required for. A few faecal pellets from mice, rats, guinea pigs, etc., can be collected from the floor of an enclosure. Similarly, a sample of dog faeces can be collected from a pen floor. If a small sample of urine is required from a dog, it may be possible to obtain it by putting a dish under the animal when it is urinating (training will help to make this technique a success). Small animals can be housed in a cage with a grid floor and a tray, without bedding material, under it.

If chemical analysis of urine and faeces is required, the samples must be preserved. Chillers are available that will cool the samples down to 2°C, which inhibits biochemical deterioration of the samples. If chillers are not available, chemical preservatives can be used, but these must be checked to ensure that they will not interfere with the subsequent analysis.

11.4.4. Measurement of food and water intake

The technique used to measure food and water intake is influenced by the accuracy required. If an approximate assessment is all that is needed, weighing the food given (a) and weighing what is left after the test period (b) and then subtracting b from a will give the weight of food consumed. If the cage has more than one occupant, the food consumed can be divided by the number of animals in the cage (n) to give the average consumption per animal.

If a more accurate measurement is required, food spill must be taken into account. Spill is the amount that falls through the hopper onto the floor, and therefore is not consumed. This can be collected by housing the animals in grid-bottomed cages with the tray lined with a paper sheet to collect the spill. The weight of the spill (c) is added to that remaining in the hopper (b) and is subtracted from the weight of the food given (a).

In summary, the formulae are as follows.

Food consumed:

food given (a) – (food left in the hopper (b) + the spill (c)) = food consumed (d):

$a - (b + c) = d$.

Average food consumed.

In a multi-occupied cage, food consumed (d) is divided by the number of animals in the cage (n) to give the average weight of food consumed per animal (e).

$d/n = e$.

A rough measurement of water consumption can be gained by recording how much the level of water in the water bottle has gone down over time, but this does not take spills into account. Measuring water spills is difficult and needs a system such as described in the next section.

11.4.5. Metabolism cages

Metabolism cages (**Figure 11.5**) are specially designed or adapted cages that allow the collection of all the urine and/or faeces produced by an animal and also enable the accurate measurement of food and water consumption over a defined period of time. Animals find these cages stressful when they are first put in them, so they should be used only when absolutely necessary, and the animals must

be acclimatised to them. This would involve putting the animals in the metabolism cage for several short periods before the real study begins. They are generally used only for 24-hour collections.

Figure 11.5. A metabolism cage

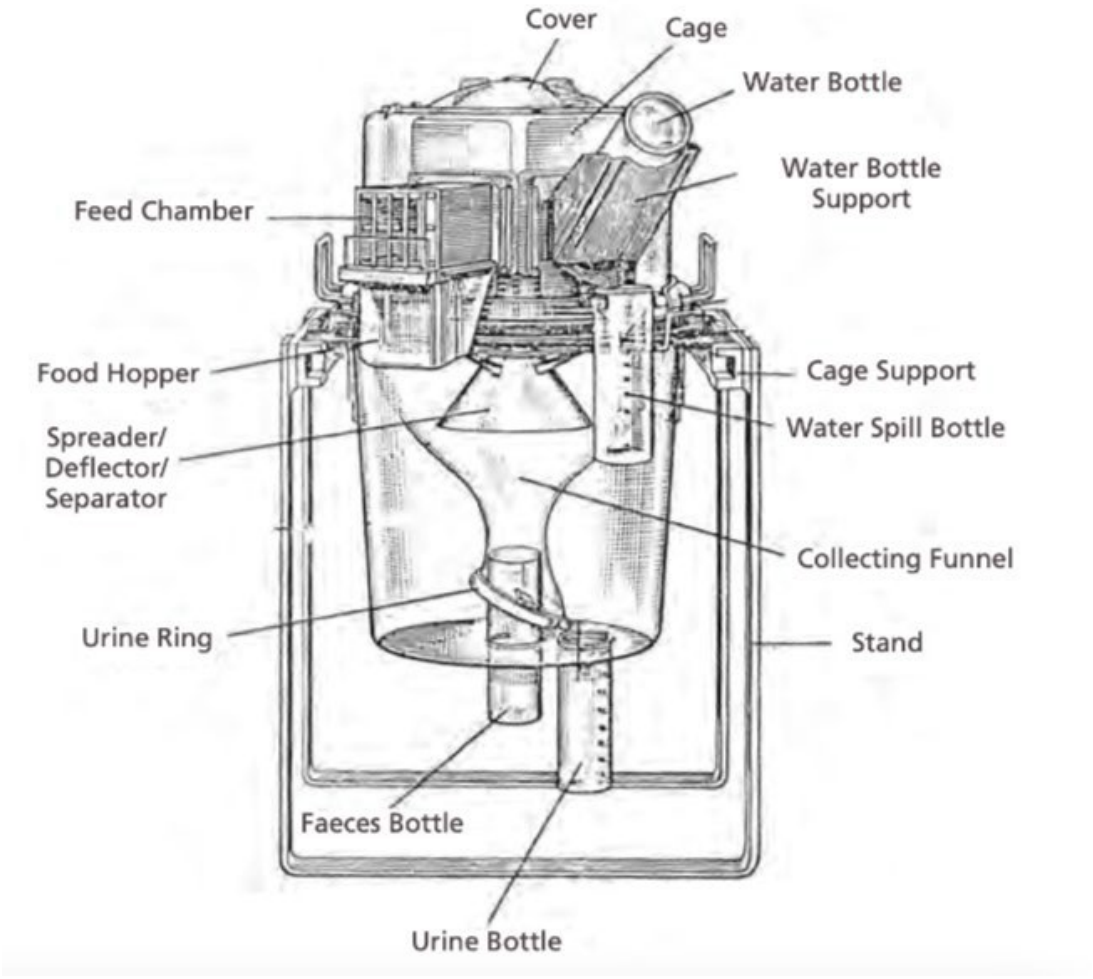
Source: Tecniplast UK

As animals find metabolism cages disturbing, an animal can be put in one only by trained staff deemed to be competent.

Figure 11.6 shows a diagram of a metabolism cage suitable for a rat or, with minor adjustments, a mouse. Any urine produced by the occupant passes through the grid bottom onto a deflector, runs off that onto the collecting funnel, where it flows down until it reaches the urine ring, which deflects the urine into the urine bottle. Faecal pellets pass through the grid and bounce off the deflector and collecting funnel to end up in the faecal bottle.

The animal has to enter a short tunnel to get access to powdered diet. This limits the risk of food contaminating the urine or faeces. The water bottle is arranged so that any spill is diverted into the spill bottle, not into the urine bottle.

Figure 11.6. Diagram of rodent metabolism cage



Source: Tecniplast

All metabolism equipment must be thoroughly cleaned to avoid contamination of the samples and to remove any odour of the previous animals. Samples of urine and faeces deteriorate quickly due to biochemical reactions within the samples and bacteriological contamination. To slow the deterioration down, chiller units are available that hold the sample bottles and cool them down. Alternatively, chemical preservatives can be used, provided that they do not affect subsequent analysis.

11.5. Post-procedural care

After undergoing procedures, all animals must be monitored according to the experimental protocol. Any adverse effect must be recorded and reported to senior staff so that remedial action can be taken as soon as possible.

Chapter 2 of this handbook explains how the severity framework of Directive 2010/63/EU (the Directive) works. It has great relevance to all procedures on living animals.

References

Council Regulation (EC) No 1/2005 on the protection of animals during transport and related operations. https://eur-lex.europa.eu/eli/reg/2005/1/oj.

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, OJ L 276, 20.10.2010, p. 33, ELI: https://eur-lex.europa.eu/eli/dir/2010/63/oj/eng.

EU, 'EUR-Lex: Access to European Union law', EUR-Lex website, https://eur-lex.europa.eu/.

NC3Rs, 'Procedures with care', NC3Rs website, https://nc3rs.org.uk/3rs-resources/procedures-care.

NC3Rs (2022), 'Blood sampling', NC3Rs website, https://nc3rs.org.uk/3rs-resources/blood-sampling.

Obernier, J. A. and Baldwin, R. L. (2006), 'Establishing an appropriate period of acclimatisation following transportation of laboratory animals', *ILAR Journal*, Vol. 47, No 4, pp. 364–369, https://doi.org/10.1093/ilar.47.4.364.

Turner, P. V., Brabb, T., Pekow, C. and Vasbinder, M. A. (2011), 'Administration of substances to laboratory animals: Routes of administration and factors to consider', Journal of the American Association of Laboratory Animal Science, Vol. 50, No 5, pp. 600–613.

Waynforth, B. (2007), 'Experimental procedures', in: Barnett, S. W. (ed.), Manual of Animal Technology, pp 351-364, Blackwell Publishing, Oxford.

Wolfensohn S and Lloyd M (1998), 'Handbook of Laboratory Animal Management and Welfare' 2nd ed., pp.75 Blackwell Science, Oxford

Further reading

Castelhano-Carlos, M. J. and Baumans, V. (2009), 'The impact of light, noise, cage cleaning and in-house transport on welfare and stress of laboratory rats', Laboratory Animals, Vol. 43, No 4, pp. 311–327.

Diehl, K.-H., Hull, R., Morton, D., Pfister, R., Rabemampianina, Y., *et al.* (2001), 'A good practice guide to the administration of substances and removal of blood, including routes and volumes', Journal of Applied Toxicology, Vol. 21, No 1, pp. 15–23, https://doi.org/10.1002/jat.727.

EU (2023), 'EU rules on the protection of animals during transport', EUR-Lex website, https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=legissum:f83007.

Contribution of this chapter to the Three Rs

Performing minimally invasive procedures on animals involves administering substances orally and by injection and withdrawing body fluids. In order to do this, the animals have to be restrained manually or in restraining devices, and sometimes they have to be put in strange environments such as metabolism cages, all of which have the potential to cause pain and distress. This chapter emphasises ways in which the techniques can be refined, before and during the procedure, to minimise the harmful effects on the animal. It also emphasises the importance of post-procedural monitoring and record-keeping and links to **Chapter 2** on the severity framework.

Self-assessment questions

1. What plans should be in place to minimise the disturbance of an animal when moving it from a holding room to the procedure room in the same facility?
2. What do you understand by the acclimatisation period?
3. What is the difference between enteral and parenteral administration routes?
4. If the total blood volume (TBV) of a 30-g mouse is 1.75 ml, what would be a safe maximum volume to take if a single sample was needed?
5. What is the purpose of a metabolism cage?

12. Euthanasia

After reading this chapter, you should be able to explain what constitutes a good death (learning outcome 6.1.1). You should also be able to describe the different methods by which laboratory animals are allowed to be killed, what affects the choice of method and how the methods can affect scientific outcomes (learning outcome 6.1.2).

12.1. Introduction

The word 'euthanasia' is derived from two Greek words: eu, which means good, and thanatos, which means death. Euthanasia therefore means a good death, and is sometimes referred to as a gentle or humane death.

A 'good death' is one in which the animal experiences minimal suffering, pain and distress, the loss of consciousness is rapid and death results before consciousness is regained.

It is the duty of all animal users to make sure that, when the need arises, the animals in their care are killed humanely. Whatever the reason for killing, individuals must be competent and confident in their ability to bring about a swift and painless death. The difference between a gentle death and a painful death can be very small, which is why Directive 2010/63/EU (the Directive) requires individuals to be judged competent by an authorised person before they are allowed to perform euthanasia.

Killing animals is an unpleasant but necessary part of working with laboratory animals, and many people find it very stressful. The culture of care, which all laboratory animal facilities should have in place (see **Chapter 3.5**), includes the care of staff, and it is important that individuals who are troubled by this or any other aspect of their work seek help from senior colleagues.

12.2. Factors that affect the choice of method of euthanasia

A number of factors need to be considered when choosing a method of euthanasia.

— Species: some species are more difficult to handle, may not have readily available veins or may react adversely to particular methods or reagents; for example, rabbits can react adversely to gases.

— Age and size: beyond a certain age and size, some physical methods (e.g. cervical dislocation) become more difficult and the likelihood of the animal suffering increases (hence the need for sedation with some methods). Difficulties may be experienced with very young animals; for example, the use of carbon dioxide is considered unsuitable for neonatal animals because it takes a very long time for them to lose consciousness and die.

— Availability of materials or apparatus: e.g. electrical stunning requires specialised equipment that few laboratory animal facilities have.

— Reason for killing: when animals are killed because a study has come to an end, the needs of the project dictate the method selected. For example, cervical dislocation would be rejected if the brain and spinal cord or the lungs were required for further study (lungs often get filled with blood following cervical dislocation). Scientists may not want chemicals contaminating tissues, which precludes using chemical means of euthanasia. If an animal has to be killed because it is injured, cervical dislocation may be rejected because of the risk of inflicting further pain and distress.

— Necessary urgency: see next section.

— Competence of the operator: see next section.

12.3. Legal considerations

The Directive requires Member States to ensure that animals being used for scientific purposes are killed with the minimum of pain, suffering and distress (Article 6, paragraph 1). It also requires that Member States ensure that, in authorised breeder, supplier and user establishments, or outside an establishment if they are part of a field study, animals are killed by a competent person (Article 6, paragraph 2). There are exceptions to this requirement, but only if scientific evidence convinces the competent authorities in the Member State that it is necessary and they grant an exemption (Article 6, paragraph 4). Article 6, paragraph 5, describes the circumstances where paragraphs 2 and 3 do not apply in emergency circumstances, for example if an animal has to be killed in emergency circumstances for animal welfare, public health, public security, animal health or environmental reasons.

Article 6, paragraph 3, and Annex IV (as amended[16]) of the Directive states the methods of euthanasia that must be used, and the species they must be used for (**Table 12.1**), in laboratory animal facilities. The methods listed can be carried out only by individuals deemed to be competent. Article 24 of The Directive states that Member States must ensure that each breeder, supplier and user appoint one or several persons who are responsible for ensuring that staff are adequately educated, competent and continuously trained and that they are supervised until they have demonstrated the requisite competence.

[16] Commission Delegated Directive (EU) 2024/1262, taking effect on 4 December 2026

Table 12.1. Methods of euthanasia to be used on laboratory animals

Animals- remarks/ methods	Fish	Amphibians	Reptiles	Birds	Rodents	Rabbits	Dogs, cats, ferrets and foxes	Large mammal	Non-Human primates	Cephalopods
Anaesthetic overdose	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	
Captive bolt			(2)							
Carbon dioxide					(3)					
Cervical dislocation				(4)	(5)	(6)				
Concussion/ percussive blow to the head				(7)	(8)	(9)	(10)			
Decapitation				(11)	(12)					
Electrical stunning	(13)	(13)		(13)		(13)	(13)	(13)		
Inert gases (Ar, N_2)								(14)		
Shooting with a free bullet with appropriate rifles, guns and ammunition			(15)				(16)	(15)		
Hypothermic shock	(17)*									

Source: Directive 2010/63/EU, Annex IV, as amended by Commission Delegated Directive (EU) 2024/1262.

Not permitted

( ) permitted subject to numbered notes below

permitted

(1)	Shall, where appropriate, be used with prior sedation.
(2)	Only to be used on large reptiles.
(3)	Only to be used in gradual fill. Not to be used for foetal or neonate rodents.
(4)	Only to be used for birds under 1 kg. Birds over 250 g shall be sedated.
(5)	Only to be used for rodents under 1 kg. Rodents over 150 g shall be sedated.
(6)	Only to be used for rabbits under 1 kg. Rabbits over 150 g shall be sedated.
(7)	Only to be used for birds under 5 kg.
(8)	Only to be used on rodents under 1 kg.
(9)	Only to be used on rabbits under 5 kg.
(10)	Only to be used on neonates.
(11)	Only to be used on birds under 250 g.
(12)	Only to be used if other methods are not possible.
(13)	Specialised equipment required.
(14)	Only to be used on pigs.
(15)	Only to be used in field conditions by experienced marksmen.
(16)	Only to be used in field conditions by experienced marksmen when other methods are not possible.
(17)	To be used only for zebrafish (Danio rerio) ≥ 16 days post fertilisation (dpf) and with a maximum body length of 5 cm. Temperature of hypothermic shock shall be ≤ 4 °C and the temperature difference from holding temperature shall be ≥ 20 °C. Fish shall not have direct contact with ice. Minimum exposure time shall be 5 minutes.

12.4. Methods of euthanasia permitted by the Directive

The intention of this section is to provide an understanding of the main methods of euthanasia permitted by Annex IV of the Directive (**Table 12.1**).

Annex IV states that animals must be sedated before some methods are used (**Table 12.1**). In these cases, a person certified as being competent to use the method of euthanasia will also be competent to sedate the animal.

Unless animals are in the same cage and are being killed at the same time (e.g. as may happen if they are being killed with carbon dioxide), animals should never be killed in the presence of other animals, as the sounds, sights and odour produced during euthanasia will be stressful and induce fear.

Animals about to be killed must be handled in the same way as at any other time so they are not aware that anything significant is going to happen to them. Animals can detect signs of distress from the scents in urine, faeces and blood left by previously killed animals, so all work surfaces, equipment and even PPE, if it has been contaminated, must be thoroughly cleaned between groups or individual animals.

Further explanation of euthanasia techniques can be found in, 'Guidelines for the euthanasia of animals,' (AVMA 2020) but this must be read alongside the instruction given in the Directive, Annex IV. Where there is a difference in views, the Directive always takes precedence.

Methods of euthanasia are divided into physical methods and chemical methods.

12.4.1. Physical methods

Physical methods are further divided into manual methods and other physical methods. The manual methods listed in paragraph 3 of Annex IV (**Table 12.1**) are cervical dislocation and concussion. Other physical methods are decapitation, captive bolt, electrical stunning and shooting with a free bullet.

Manual methods

The high level of skill required to use these methods is gained by practice on dead animals that have been killed by chemical means so that their necks are not damaged.

Dislocation of the neck (cervical dislocation)

— **Mouse**. An adult mouse may be killed by holding it by the base of the tail and allowing the front feet to grip a suitable surface such as a cage top. The finger and thumb are then run up the body in the way similar to that used to scruff the animal. At the base of the skull, the thumb and first finger are quickly pushed forward and down and, at the same time, while maintaining tension, the tail is raised to a 45-degree angle to produce the dislocation.

— **Rat**. A variation of the technique described for mice may be applied to newly weaned rats (also called post weaner rats). It is often advisable to grip the hind legs and the tail. Rats with a body weight of over 150 g must be sedated before dislocation of the neck using the thumb and forefinger method described for the mouse. When the rat is sedated, an implement such as a bar placed across the head could be used instead of the thumb and forefinger.

— **Guinea pig**. Dislocation of the neck can be used for guinea pigs, but if the animal is over 150 g a sedative or anaesthetic must be used. The guinea pig is placed on the bench and turned to face the operator. A hand is placed over its head and the first and second fingers are slid down the sides of the neck and are bent under the angles of the jaw. The guinea pig is lifted clear of the bench and the body is swung down rapidly, 'flicking' the wrist. The weight of the body dislocates the neck.

— **All rodents**. Cervical dislocation cannot be used to kill rodents over 1 kg body weight, and rodents over 150 g must be sedated.

— **Rabbit**. Rabbits up to 1 kg body weight can be killed with the 'rabbit punch'. If they are over 150 g they must be sedated or anaesthetised. The rabbit is taken from the bench and suspended in one hand by the back legs. The other hand is used to deliver a 'karate chop' onto the base of the skull behind the ears. Some operators prefer to use a suitable heavy stick instead of the hand.

— **Birds**. Chickens are used as an example here; different techniques are required for different birds. Cervical dislocation can be used only for birds under 1 kg. Birds over 250 g must be sedated. The legs of the chicken are taken in one hand with the arm around the body of the bird. The first two fingers of the other hand go over the back of the head and under the beak. The second hand pulls the neck down while directing the beak upwards. This breaks the spinal cord and separates the vertebrae in the cervical region. The gap between the vertebrae can be felt by palpating (feeling) the neck.

Concussion (stunning by striking the back of the head)

Concussion causes unconsciousness quickly, but does not necessarily cause death. The method can be used for rodents and rabbits under 1 kg; rabbits under 5 kg; neonate dogs, cats, ferrets and foxes; and birds under 5 kg.

The technique varies depending on species and age. The technique for a rat is as follows: using one hand to hold the base of the tail and/or hindquarters, the rat is lifted, turned over and swung down sharply so that the base of the skull strikes a solid object such as the sharp edge of the bench.

This method only stuns, death must be confirmed by one of the methods listed in **Chapter 12.5**.

Other physical methods

Decapitation

This method is allowed only on birds under 250 g, and on rodents when no other methods are possible. The method may be suitable when a study seeks to measure physiological parameters that may be affected by other methods, for example injectable or inhalation agents. A purpose-made guillotine must be used and the animals must be securely immobilised during the procedure, often by placing the animal in a plastic sleeve. The guillotine must be kept clean, well lubricated and sharp.

Severance of the neck should cause rapid failure of the cerebral circulation, resulting in the loss of consciousness.

Captive bolt gun

Captive bolt guns come in several different designs; a typical one resembles a pistol but, instead of a bullet, it fires a blank cartridge (see **Figure 12.1**). The muzzle of the gun is placed directly on the head of the animal; exactly where it is placed depends on the species of animal. When the trigger is

squeezed, a captive bolt is projected into the brain. The degree of penetration depends on the strength of the blank cartridge, which, in turn, depends on the size of the animal.

Figure 12.1. Captive bolt gun

Source: Stephen Bernett

This method only stuns, so, to ensure death it must be quickly followed by one of the methods listed in **Chapter 12.5**. Captive bolt guns can be used to stun large reptiles, rabbits and large mammals.

Free bullets

Free bullets can be used only on reptiles and large animals in field conditions, and on dogs, cats, ferrets and foxes in field conditions when other methods are not possible. In all cases they can be used only by an experienced fire arms expert.

Guns using free bullets are obviously very dangerous – not only when the bullet leaves the gun but also when it enters the body, as there is no certainty where it will exit the body. Even small bones or bone fragments can divert it from its trajectory and cause it to exit in unexpected directions. The only safe place to be when animals are being killed with free bullets is behind the shooter.

12.4.2. Chemical methods

Overdose of anaesthetic by injection

Several drugs are available to produce euthanasia by injection. The most often used routes are intravenous, in animals with readily available veins, and intra-peritoneal, for those without easily accessed veins.

The most suitable drug to use in any situation will depend on the age and species of the animal and the experimental requirements. One commonly used drug is sodium pentobarbitone, which produces almost instantaneous loss of consciousness and subsequent death when administered by the intravenous route. Injections must be given relatively quickly in order to overcome the excitation phase

that occurs with these drugs. It is also important to ensure that the needle is completely in the vein before injecting sodium pentobarbitone as it has a high pH and causes irritation if it is injected into surrounding tissues. For the same reason, it is not suitable for the intra-peritoneal route.

Overdose of anaesthetic by inhalation

Methods involving inhalation of anaesthetic gases may be unacceptable for some experimental work, as lung tissues may be damaged and blood gas levels will be changed. They should always be administered with oxygen. When appropriate, prior sedation should be used.

Anaesthetic gases do present a hazard to those administering them and should be used only if an efficient scavenging system is in operation.

Euthanasia by inhalation using any gas is not recommended for animals aged less than 10 days, as they take a very long time to die. Animals over 1.5 kg should not be killed by any inhalation gas administered in a chamber, as they too take a long time to die, and they panic.

Any animal that is undergoing an experimental procedure under inhalation anaesthesia, and is unconscious, may be killed by an overdose of the anaesthetic.

Carbon dioxide

Carbon dioxide is the most commonly used gas for rodents from 10 days old to 1.5 kg. An animal exposed to carbon dioxide in a rising concentration will fairly quickly fall into a state of unconsciousness. Great care needs to be taken when killing animals with this carbon dioxide, as discomfort to the animal is impossible to eliminate completely, but carelessness can turn a relatively minor discomfort into significant pain.

Carbon dioxide should be administered in purpose-built chambers that allow a regulated flow of the gas. The chamber should start by being filled with normal environmental air. The chamber volume should be calculated and the flow rate of the carbon dioxide set so that 20% of the chamber volume will fill per minute. This will minimise agitation or panic (AVMA, 2020). Such chambers allow a steady build-up of carbon dioxide without causing noise from the cylinder or a rapid change of temperature, which would distress the animals (**Figure 12.2**).

Figure 12.2. A euthanasia cabinet for use with carbon dioxide

Source: Stephen Barnett

The chamber must be cleaned between each group of animals being killed, and the chamber should be free from residual carbon dioxide, as immediate exposure to high levels of carbon dioxide (over 50%) results in panic and pain in animals (Hawkins *et al*., 2016). Emptying carbon dioxide from the chamber is straightforward because the gas is heavier than air, so just inverting the chamber will cause it to pour out, or, if there is a tray fitted inside, lofting it out will have the same effect. To refine this process further, systems are available whereby the home cage can be placed directly into the carbon dioxide chamber to reduce stress (**Figure 12.3**).

Figure 12.3. A carbon dioxide euthanasia system designed to hold a specific cage

Source: VetTech Solutions

12.5. Recognition of signs of death

Signs that indicate that an animal has died include lack of movement, lack of breathing, lack of reflexes, lack of heartbeat, lack of blood pressure, change of skin colouration to a blue tinge and involuntary urination or defecation.

It is unsafe to assume that the presence of any of these indicators necessarily means that the animal has died.

Death must be completed by one of the following methods, in bold type (Annex IV).

— **Dislocation of the neck**: when a small animal has been stunned or when it has been killed with an injectable or inhalation gas, cervical dislocation will confirm death.

— **Exsanguination**: in this technique, a major vessel is severed so that the animal bleeds to death. It is a suitable method to use after stunning techniques.

— Confirmation of **permanent cessation of circulation**: this is a sure sign of death.

— **Destruction of the brain**: the technique is called pithing. For instance, following stunning with a captive bolt gun, a rod or spring is put into the hole made by the bolt and stirred around to destroy the brain.

— Confirmation of the onset of **rigor mortis**: stiffening of the skeletal muscles that occurs after death may be taken as conclusive evidence of death.

References

AVMA (American Veterinary Medical Association) (2020), 'Guidelines for the euthanasia of animals: 2020 edition', AVMA website, https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf.

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, OJ L 276, 20.10.2010, p. 33, ELI: https://eur-lex.europa.eu/eli/dir/2010/63/oj/eng.

Hawkins, P., Prescott, M. J., Carbone, L., Dennison, N., Johnson, C., *et al.* (2016), 'A good death? Report of the second Newcastle meeting on laboratory animal euthanasia', *Animals*, Vol. 6, No 9, p. 50, https://doi.org/10.3390/ani6090050.

Further reading

Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing, OJ L 303, 18.11.2009, p. 1, ELI: http://data.europa.eu/eli/reg/2009/1099/oj.

Contribution of this chapter to the Three Rs

The major contribution of the Three Rs to euthanasia is in the area of refinement, but there is also a small contribution to relative replacement.

Refinement. Animals must always be treated with kindness and respect, and never more so than when their lives are coming to an end. Lack of concentration will turn a situation that should be painless and only mildly aversive into a painful and frightening one. The following are important points to remember.

— The animals must be treated exactly the same when they are going to be killed as at any other time.

— Animals are able to sense if their fellows have been killed, so killing should not take place in the same room as living animals. Operators must make sure all body fluids or any other signs of death have been removed from their hands, protective clothes, the inhalation chamber (if it is being used), any other equipment used and the general environment.

— Only competent people should carry out euthanasia. Within the constraints of the law and the scientific demands, attempts should constantly be made to seek to refine the methods used.

— When equipment is being used, it must be checked before the procedure commences, to ensure that it works correctly, there is sufficient gas, etc.

Relative replacement. When possible, attempts should be made to use tissues from the animal immediately or after preservation, for studies using alternatives to live animal techniques. This saves other animals from being killed for that purpose.

Self-assessment questions

1. What constitutes 'a good death'?
2. Who can perform euthanasia in laboratory animal facilities in the EU?

3. Where can the permitted methods of euthanasia and the species they can be used on be found?
4. After stunning an animal, what methods can be used to ensure that death follows?
5. List two sure signs that an animal is dead.

13. Handling and sexing

After reading this chapter, you should be able to list the methods and demonstrate an understanding of the appropriate, safe and humane handling, sexing and restraint of one or more named species for common scientific procedures (learning outcome 4.7).

You should also be able to describe the consequences of habituation and training (learning outcome 4.4).

13.1. Introduction

All individuals who work with laboratory animals must be able to handle, sex and restrain them while causing the minimum amount of stress or harm. In addition to ensuring that the animals are not harmed, the method used must be appropriate for the purpose and should not put the handler at risk of injury. This chapter describes methods of handling and sexing that are in common use, but the level of skill expected of those handling animals cannot be gained by reading alone. Competence in animal handling can be achieved only by practice under the supervision of a more experienced colleague.

Handlers are required to wear Personal Protective Equipment (PPE), including gowns, gloves, masks, hats and boots. This limits exposure to Laboratory Animal Allergens (LAA) for handlers and reduces the risk of pathogens being transferred between animals and handlers, and from handlers to other animals.

13.2. General principles of animal handling and sexing

All laboratory animals get very anxious when they are handled for the first time or when handled by an unfamiliar handler, and they will attempt to avoid the experience. This is an inborn (or innate) response, but it can be overcome if their experience of handling is a positive one and they realise their avoidance response is unnecessary. This pattern of behaviour is called habituation. Physiologically, habituation reduces unnecessary responses to stimuli that are not harmful so that the animal can save resources for when avoidance really is necessary. It follows that a bad handling experience will confirm the wisdom of the initial avoidance response.

Anxiety in animals is an important welfare issue, and it adversely influences scientific results, so it is essential that the risk of it is minimised.

In order to reduce anxiety, time should be spent getting to know the animal and letting the animal get to know the handler before handling commences. This is particularly important when animals are handled for the first time by a new handler or a new handling technique; animals identify and trust individual handlers, and new handlers have to gain their confidence. Spending time with young animals (socialising) makes subsequent handling much less alarming for them. Animals should always be approached in a confident and relaxed manner, there should be no sudden movements and they should be calmed by speaking to them in a gentle voice.

Restraint may be necessary for some procedures, but it should always be the minimum possible to perform the procedure safely. Before it is carried out for the first time, the handlers should make sure that they have the animal's confidence and that the animals are used to be being picked up and stroked, etc. The restraint technique can then be introduced, at first without the procedure being carried out.

Complex procedures are made less worrying for an animal if it undergoes a period of preparatory training. Positive reinforcement training is an important method of reducing the need for excessive restraint.

Positive reinforcement training is a process whereby animals are taught to perform a particular behaviour: when they perform it, they are given a reward, usually in the form of a food treat. When the desired behaviour has been carried out, it is often indicated to the animal immediately by a sound such as a click or a whistle. This type of training has had a variety of uses, for example training non-human primates to offer their arms out of their cages so blood samples can be taken, training dogs or pigs to sit on a weighing machine or enter a trolley so they can be wheeled to another room, and training rats so that blood samples can be taken without restraint. See Laule (2010) for an overview of positive reinforcement training, and see the video 'Housing, handling and training rats at RISE', produced by the Research Institutes of Sweden (RISE), (https://3rc.org/rats-refinement).

Sexing

Distinguishing the sex of adult animals is usually obvious. In young animals where the external sex organs are not so obvious, the anogenital distances are the main criteria for determining the sex of an individual. There is a longer distance between the anus and the prepuce of the male than between the anus and genital opening of the female (see **Chapter 13.3** for illustrations).

13.3. Techniques for individual species

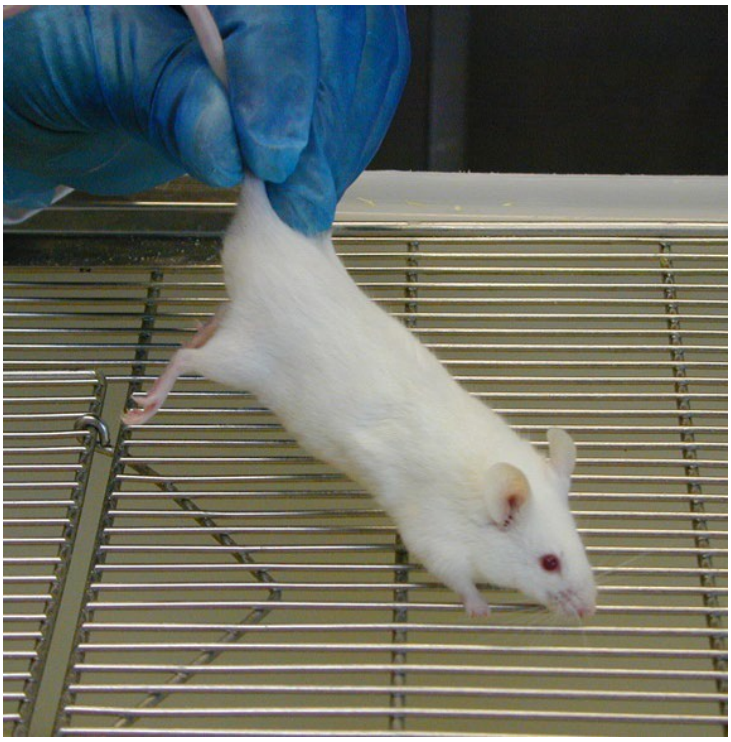
The animals covered in this chapter are mice, rats, Syrian hamsters, guinea pigs, rabbits, ferrets, cats, dogs and zebrafish.

13.3.1. The mouse

Handling

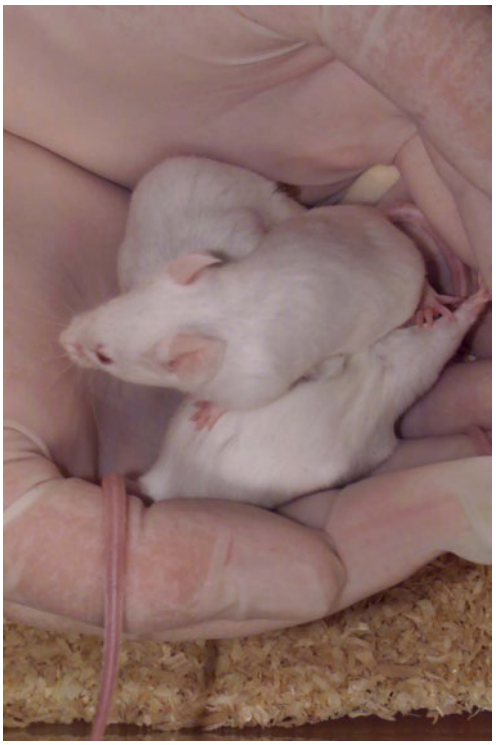
Adults. The traditional method of picking up a mouse is by the base of the tail and immediately supporting its weight on the other hand or on another surface (**Figure 13.1**). Tail handing is thought by some to increase anxiety in mice (Hurst and West, 2010) but others believe tail handling is still an acceptable method (Meyer *et al* 2025). Cupping (**Figure 13.2**) or using a plastic tube (**Figure 13.3**) are other available methods dependent on the purpose for the handling. Cupping mice is useful for very young animals and lifting groups of mice during cage changing. Using tubes and cupping to pick up mice is demonstrated online (https://www.nc3rs.org.uk/mouse-handling-video-tutorial).

Figure 13.1. Picking up a mouse by the tail



Source: Stephen Barnett

Figure 13.2. Moving mice by cupping in the hand



Source: IAT

Figure 13.3. A mouse being guided into a plastic tube

Source: John Waters

Pregnant females must be scooped up bodily using both hands, taking care not to squeeze them.

As demonstrated in the RISE video, mentioned previously, mice can be trained to undergo some procedures without restraint, but, when necessary, a mouse may be restrained by picking it up by the base of the tail and allowing it to grip a rough surface (e.g. a cage top). It will pull away from the handler. The scruff of the neck and loose skin over the shoulders may then be grasped with the thumb and fingers (**Figure 13.4**) and the hand turned so that the mouse lies on its back. The tail can be restrained with a little finger.

Figure 13.4. Restraining a mouse

Source: IAT

Very young mice. Newborn mice are gently picked up bodily; several may be scooped up at a time.

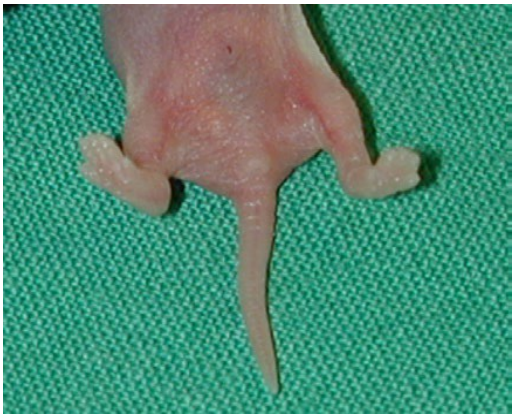
Weaners. When mice are about three weeks old, they become very active and may need to be held by the scruff of the neck or base of the tail in order to control them.

Sexing

— Very young mice. The pups are held so that the genitalia can be inspected and the anogenital distances can be compared. When body hair begins to grow, the region surrounding the teats in the female remains hairless until approximately 16 days of age. This can help identify females (**Figure 13.5**, **Figure 13.6**, **Figure 13.7**, **Figure 13.8**, **Figure 13.9**, **Figure 13.10**).

— Weaners. Anogenital distances are used to distinguish between the sexes until about six weeks of age, when the testes descend in the male. From then on, males and females are easily distinguished.

Figure 13.5. A one-day-old female mouse



Source: Stephen Barnett

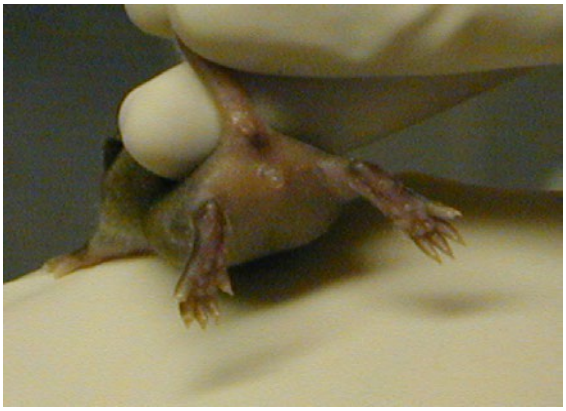
Figure 13.6. A one-day-old male mouse

Source: Stephen Barnett

Figure 13.7. A 10-day-old female mouse

Source: Stephen Barnett

Figure 13.8. A 10-day-old male mouse

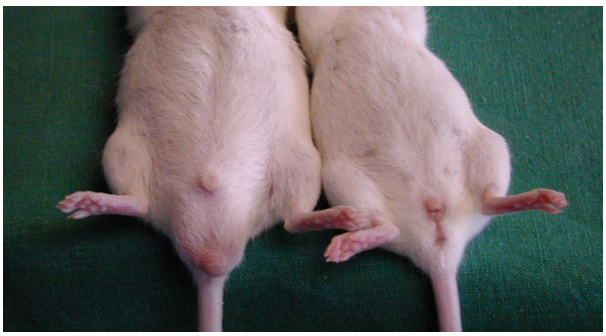


Source: Stephen Barnett

Figure 13.9. Weaner mice, female (left) and male (right)

Source: Stephen Barnett

Figure 13.10. Adult mice, male (left) and female (right)



Source: Stephen Barnett

13.3.2. The rat

Handling

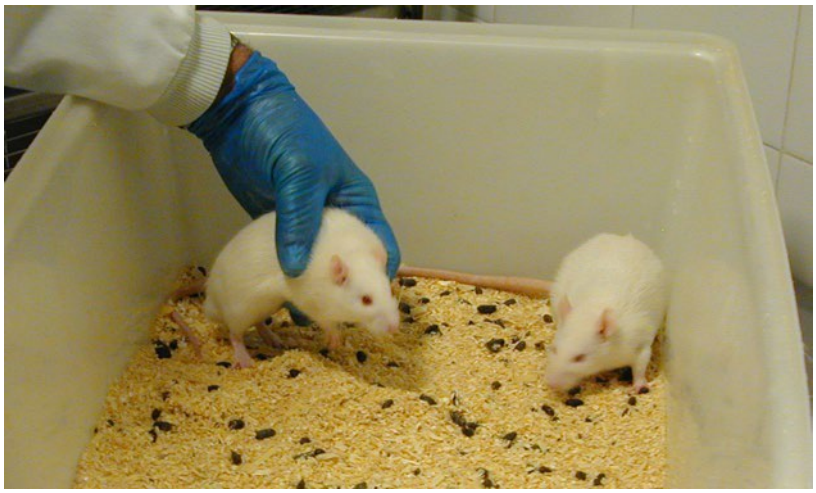
In addition to the preparatory habituation techniques, which have already been mentioned, anxiety in rats when being handled can be diminished by a technique called rat tickling. Essentially this involves mimicking the tumbling and wrestling that form the play of young rats (Cloutier *et al*., 2018).

Adults. Adult rats are picked up by placing the thumb and forefinger around the neck with the palm of the hand across the shoulders; the remaining fingers are placed around the chest to assist when lifting the animal (**Figure 13.11**). Larger rats must be supported by placing the free hand under the body when lifting. Small groups of young rats can be transferred from one cage to another by scooping them up with both hands.

The RISE video (see above) shows how trained rats are willing to undergo procedures such as blood collection with minimum restraint.

If restraint is necessary, adult rats can be restrained by gently increasing the finger and thumb pressure around the neck and using the thumb to control the jaw (**Figure 13.12**). The hind legs and/or tail must be supported and restrained by the other hand.

Figure 13.11. Picking up a rat



Source: Stephen Barnett

Figure 13.12. Restraining a rat

Source: Stephen Barnett

Very young rats. Pups should be handled in the way described for mice of a similar age, but, by the time they are two weeks old, the best method is to pick them up boldly, as, at this stage, the skin covering their tails is extremely delicate.

Sexing

Rats are sexed in the same way as mice, but, as the animals are larger, the anogenital distance and genitals are also proportionately larger (**Figure 13.13**, **Figure 13.14**, **Figure 13.15**, **Figure 13.16**, **Figure 13.17**, **Figure 13.18**, **Figure 13.19**, **Figure 13.20**).

Figure 13.13. Female rat, one or days old

Source: Stephen Barnett

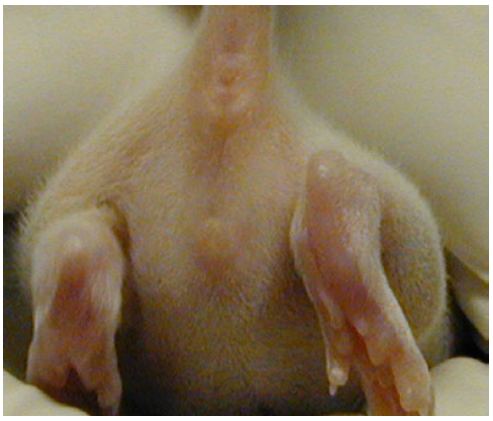
Figure 13.14. Male rat, one or two days old

Source: Stephen Barnett

Figure 13.15. Female rat, 10 days old

Source: Stephen Barnett

Figure 13.16. Male rat, 10 days old



Source: Stephen Barnett

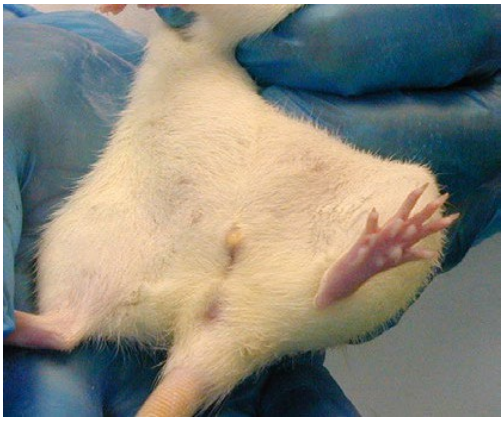
Figure 13.17. Female rat, 19 days old

Source: Stephen Barnett

Figure 13.18. Male rat, 19 days old

Source: Stephen Barnett

Figure 13.19. Adult female rat



Source: Stephen Barnett

Figure 13.20. Adult male rat

Source: Stephen Barnett

13.3.3. The Syrian hamster

Hamsters are nocturnal and are much more likely than mice and rats to be found asleep during the daytime. When disturbed in these circumstances, they may roll onto their backs and vocalise their disapproval. Hamsters should be allowed to wake up fully before any attempt is made to pick them up, as they will be much calmer.

Handling

Hamsters may be scooped up with one or both hands (**Figure 13.21** and **Figure 13.22**) or picked up by the scruff. If it is necessary to restrain the animal, a hind leg can be held between the fingers. This technique requires much more practice than that used for mice.

Figure 13.21. Cupping a hamster

Source: IAT

Figure 13.22. Picking up a hamster

Source: Stephen Barnett

Sexing

Adults. A hamster can be sexed while it is kept in the hand by rolling it onto its back and turning the top hand so that the genital region is exposed. When adult male hamsters are upturned, the testes and scrotal sacks are often not as obvious as in mice and rats, but are obvious when the animal is moving in the cage. An initial impression of the sex of an adult, without handling it, can be gained by observing the shape of the rear end of the body when the animal is in its cage (**Figure 13.23** and **Figure 13.24**). Young and immature hamsters can be sexed in the way previously described for mice of the same age.

Figure 13.23. Male hamster

Source: Stephen Barnett

Figure 13.24. Female hamster

Source: Stephen Barnett

13.3.4. The guinea pig

Guinea pigs are easily excited and become difficult to catch and handle if they are disturbed. So it is particularly important to approach them calmly and quietly.

Handling

Guinea pigs are picked up in the same way as previously described for the rat. The thumb and forefinger are placed around the neck with the palm of the hand across the shoulders; the remaining fingers are used to assist when lifting the animal (**Figure 13.25**). Larger guinea pigs should be supported at the rear end with the free hand. Extreme care should be taken when handling late-term pregnant females. They may be lifted up after sliding one hand under the body between the four legs and the other hand under the body between the hind legs.

Figure 13.25. Holding a guinea pig

Source: Martin Heath

Sexing

The shape of the genital area of guinea pigs varies between the sexes: the female genital region is described as Y-shaped and the male region is described as a line with a dot (**Figure 13.26**, **Figure 13.27**, **Figure 13.28**, **Figure 13.29**). If gentle pressure is applied in front of the genital region of the male, the penis will be expressed, but this is not necessary in order to tell the sexes apart.

The teats are no guide to the sex of the guinea pig, as they are well developed in both sexes.

Figure 13.26. Adult female guinea pig

Source: Stephen Barnett

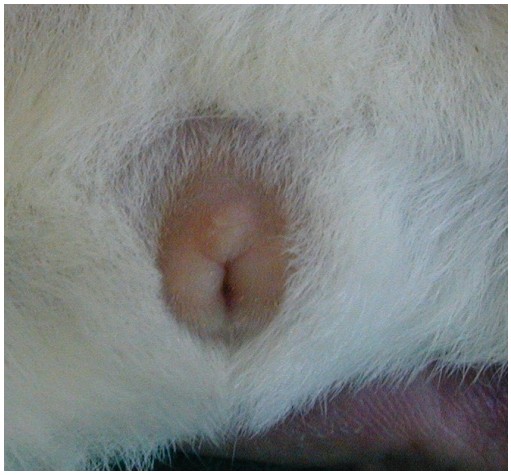
Figure 13.27. Adult male guinea pig

Source: Stephen Barnett

Figure 13.28. Immature female guinea pig

Source: Stephen Barnett

Figure 13.29. Immature male guinea pig



Source: Stephen Barnett

13.3.5. The rabbit

Handling

If rabbits are handled badly, they can twist their backs, which may result in spinal damage. The hindlimbs are very powerful and the claws can also inflict serious damage on the handler. When a rabbit needs to be transferred from place to place or cage to cage (**Figure 13.30**), it can be trained to enter a carrying box, so it does not need to be handled at all, thereby lessening the anxiety of the process (Kinally *et al.*, 2023).

Figure 13.30. Rabbit about to be put into a transport box

Source: Kinally, Onions and Glenn (2023)

A rabbit can be safely removed from its cage by holding the skin of the scruff of the neck. The second hand can control the hindlimbs by either placing the hand behind the animal so it can be bunched up slightly (**Figure 13.31**) or sliding the hand under the rabbit so that, as it is lifted, the back is straightened and the hindlimbs are extended slightly (**Figure 13.32**).

Figure 13.31. Removing a rabbit from the cage

Source: Stephen Barnett

Figure 13.32. Rabbit in a carrying position

Source: Martin Heath

If a rabbit is to be carried any distance and it is not possible to use a carrying box, it can be transferred to the chest of the handler without releasing the scruff, and the second hand can be placed under the hindquarters to support the weight of the body (**Figure 13.33**). Alternatively, the rabbit's head can be tucked under the arm of the handler, covering its eyes, and the body well supported. When returning a rabbit to its cage, it is essential to prevent it from kicking with its hindlimbs; this is done by returning it rear end first.

Sexing

An immature rabbit can be sexed by picking it up by the scruff of the neck while supporting the body with the second hand. The second hand can also be used to hold the tail back, and the thumb can be used to clear the fur around the genitalia (**Figure 13.33**). Slight pressure applied above the genital region will expose the vaginal opening as a slit, in the female and the rudimentary penis, with its round opening, in the case of a male (**Figure 13.34** and **Figure 13.35**). Mature rabbits may need to be supported on a table or the thigh of the handler when sexing. The sex difference is clear in adults; in the male, the penis and testicles can be clearly seen and in the female, the vaginal opening and vulva are more obvious (**Figure 13.36** and **Figure 13.37**).

Figure 13.33. Holding a rabbit for sexing

Source: Stephen Barnett

Figure 13.34. Immature female rabbit

Source: Stephen Barnett

Figure 13.35. Immature male rabbit

Source: Stephen Barnett

Figure 13.36. Adult female rabbit

Source: Stephen Barnett

Figure 13.37. Adult male rabbit

Source: Stephen Barnett

In larger breeds and older animals, secondary sexual characteristics may be apparent; the female may have a dewlap and a fatty pad under the jaw, and the male is generally more thickset with a squarer head.

13.3.6. The ferret

Handling

Ferrets can be easily tamed with regular, gentle handling. They are very inquisitive and will come to the open doors of their cages and extend their necks in order to see out. It is useful to distract the ferret with one hand while the other hand is used to grasp its neck, using the thumb to control the lower jaw as if it were a rat. The hindquarters should be supported on lifting (**Figure 13.38**). The ferret has a long back, which should be kept straight; often the forearm is used to support the body. If the ferret is being approached from above, it may be necessary to restrain the animal by the tail before grasping it by the neck.

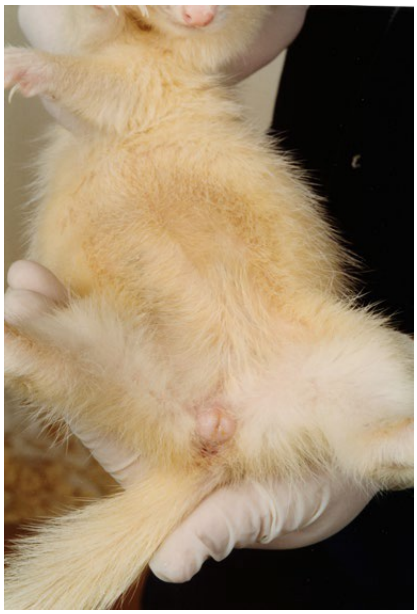
Figure 13.38. Holding a ferret

Source: Martin Heath

Sexing

By the inexperienced, the swollen vulva of the female in oestrus may be mistaken for a scrotum. The position of the genitalia is similar to that in the dog (**Figure 13.39** and **Figure 13.40**). The body weight of adult ferrets varies considerably through the season.

Figure 13.39. Female ferret



Source: Martin Heath

Figure 13.40. Male ferret

Source: Martin Heath

13.3.7. The cat

Handling

Many people are frightened of the sharp claws and teeth of cats, but laboratory cats should be sufficiently tame for them to be picked up bodily in a way similar to that used for a pet cat. Time spent petting them ensures that cats will remain happy to be handled. Cats can be transported in a carrying box. A technique similar to that used for a rabbit may be employed to remove a cat from the cage.

Sexing

The size and shape of the head and body can be used as a guide to sexing mature cats; the males tend to be larger and squarer. Inspection of the genital region will reveal a clear difference between the sexes in both kittens and adults (**Figure 13.41**, **Figure 13.42**, **Figure 13.43**, **Figure 13.44**).

Figure 13.41. Female kitten

Source: Stephen Barnett

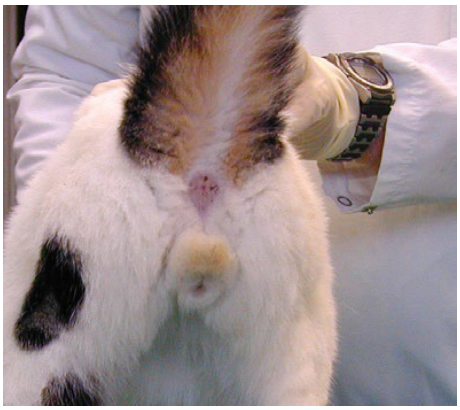
Figure 13.42. Male kitten

Source: Stephen Barnett

Figure 13.43. Adult female kitten

Source: Stephen Barnett

Figure 13.44. Adult male kitten



Source: Stephen Barnett

13.3.8. The dog

Handling

Breeds of dogs vary considerably in size; therefore, techniques for handling need to be adapted accordingly. Handling for the beagle, a medium-sized and commonly used laboratory dog, is illustrated here. The confidence of the dog should initially be gained by eye contact and talking to it. Squatting down to approach the dog from its level can help to keep it calm. It can then be picked up bodily (**Figure 13.45**), but be aware of the weight (see **Section 14.5**). Dogs can be trained to perform any tasks in the facility, for example walking on a lead and walking up a ramp onto a table. Training and habituation greatly reduce stress in the dog and the handler.

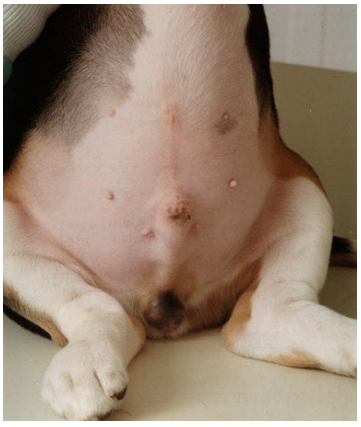
Figure 13.45. Lifting a male dog

Source: Martin Heath

Sexing

Inspection of the genital region will reveal a clear difference between the sexes in both puppies and adults. The penis and scrotal sac are usually obvious without picking the dog up (**Figure 13.46**, **Figure 13.47**, **Figure 13.48**).

Figure 13.46. Male puppy



Source: Martin Heath

Figure 13.47. Female puppy

Source: Martin Heath

Figure 13.48. Adult female (bitch)

Source: Martin Heath

13.4. Example of a non-mammalian species: zebrafish by Nicola Goodwin

13.4.1. Handling

Adults

The most commonly used method to transfer zebrafish from one tank to another is with a net. Care must be taken to ensure that the net is of a soft nylon mesh to avoid injury. Fish should be encouraged to enter the net with minimal handling and released back into the water as quickly as possible to avoid a stress response. To ensure good hygiene within the facility, the net should be cleaned between uses. When chemicals are used to perform the cleaning process, a thorough rinse procedure must be included to avoid the system becoming contaminated.

Fry

Individual fry may be handled using a small net with a very fine mesh (a tea strainer is commonly used as an alternative). The fry should be released into a new tank by placing the net within the system water, allowing the fry to swim away without the requirement of manual removal. Groups of fry may be moved by gently pouring them from one tank to another, ensuring that the tank they are to enter contains a sufficient depth of water to avoid the fry touching the bottom of the tank during pouring.

Restraint

Following anaesthesia, adults and juveniles can be restrained using a piece of soft damp sponge. A small groove cut within the sponge can allow for the fish to be restrained with either the dorsal or the ventral surface exposed (**Figure 13.49**).

Figure 13.49. Zebrafish restrained on a sponge

Source: Nicola Goodwin

13.4.2. Sexing

The time at which zebrafish become sexually mature can vary from two to six months, depending upon environmental conditions, stocking density and diet provisions. Because of this, size and appearance are considered more appropriate ways to determine sexual maturity than age. Zebrafish are sexually dimorphic, allowing the sex to be determined through observation without the requirement of handling. Males (**Figure 13.50**, top) have dark blue stripes, a streamlined body shape (torpedo shaped), are typically more active than females, have a pinkish yellow contrast, have a golden colour on the ventral fins and can have longer anal fins than females. The females (**Figure 13.50**, bottom) have a silver-white protruding belly, have a bluish-white contrast, have silver stripes between the blue and are typically of larger body size than males of the same age.

Figure 13.50. Adult zebrafish male (top), female (bottom)

Source: Nicola Goodwin

References

Cloutier, S., LaFollette, M. R., Gaskill, B. N., Panksepp, J. and Newberry, R. C. (2018), 'Tickling, a technique for inducing positive affect when handling rats', *Journal of Visualized Experiments*, Vol. 135, e57190, https://doi.org/10.3791/57190.

Hurst, J. L. and West, R. S. (2010), 'Taming anxiety in laboratory mice', *Nature Methods*, Vol. 7, pp. 825–826, https://doi.org/10.1038/nmeth.1500.

Kinally, A., Onions, L. and Glenn, S. (2023), 'Validating the use of box training as a refinement to rabbit breeding', Animal Technology and Welfare, Vol. 22, No 1, pp. 35–40.

Laule, G. (2010), 'Positive reinforcement training for laboratory animals', in: Hubrecht, R. and Kirkwood, J. (eds), The UFAW handbook on the care and management of laboratory and other research animals, 8th edition, Wiley-Blackwell, Chichester, pp. 206–218.

Meyer N, Gottschalk R, Jakobi L, Schonhoff A-M and Touma C (2025) Nature Scientific Reports https://www.nature.com/articles/s41598-025-07384-w.

NC3Rs (2018), 'Mouse handling: Tutorial', NC3Rs website, https://www.nc3rs.org.uk/mouse-handling-video-tutorial.

Research Institute of Sweden, Housing, handling and training rats at RISE. https://3rc.org/rats-refinement.

Contribution of this chapter to the Three Rs

- Handling and restraint provide the opportunity to support the three Rs in the area of refinement.
- Giving animals the opportunity to get used to the handler reduces the innate fear that would otherwise ac-company being handled, in a process called habituation.
- Training can reduce the need for close restraint for many procedures, and can provide enrichment for the animals.
- Accurately sexing animals prevents unwanted births and the possibility of fights when males of aggressive strains are put together by mistake.
- Rough handling and restraint not only induce fear but also risk injuring the animal. Becoming proficient in handling and sexing makes the experience less stressful for both the animal and the carer.

Self-assessment questions

1. Explain how to reduce the anxiety suffered by mice when first handled.
2. Describe two methods of transporting rabbits from one location to another.
3. Outline the technique that can be used to reduce the need for close restraint with rats undergoing simple procedures.
4. What anatomical features allow male and female mice to be distinguished at two days of age?
5. Describe how a zebrafish can be moved from one tank to another.

14. Safety and health in the laboratory animal facility

After reading this chapter, you should be able to identify human health hazards associated with working in laboratory animal facilities, particularly allergy, infection, injury and zoonosis, and how these can be prevented (learning outcome 4.13).

14.1. Introduction

Laboratory animal facilities present risks to the safety and health of people working in them. If animals, effluent or polluted air escape, people working outside the facility, and even the general public, may be affected. The risks are minimised by following the employer's safety and health policy and practices; in fact it is a legal requirement to do so. This chapter is designed to provide a basic understanding and awareness of safety and health issues.

14.2. Legal considerations

The EU has the aim of 'Making Europe a safer, healthier and more productive place to work' (European Agency for Safety and Health at Work 2017). To achieve this, it has instituted a number of directives and regulations on safety and health issues. The first of these was the occupational safety and health (OSH) framework directive (Framework Directive 89/391/EEC).

The OSH framework directive states the minimum safety and health requirements at work, but allows Member States that have established higher standards to maintain them, and encourages all Member States to work towards higher standards.

The OSH framework directive places an obligation on employers to ensure the safety and health of workers in every aspect related to work. Employers must:

— evaluate all the risks to the safety and health of workers;

— implement measures that assure an improvement in the level of protection afforded to workers;

— take into consideration a worker's capabilities when entrusting them with tasks;

— consult workers on the introduction of new technologies;

— designate workers to carry out activities related to the protection and prevention of occupational risks;

— take the necessary measures for first aid, firefighting, evacuation of workers and action required in the event of serious and imminent danger;

— keep a list of occupational accidents and draw up reports for responsible authorities on occupational accidents experienced by workers;

— ensure that each worker receives adequate safety and health training.

Obligations are also placed on workers to:

— make correct use of machinery, apparatus, tools, dangerous substances, transport equipment, other means of production and PPE;

— immediately inform the employer of any work situation presenting a serious and immediate danger and of any shortcomings in the protection arrangements;

— cooperate with the employer in fulfilling any requirement imposed for health and safety and to ensure that the working environment and working conditions are safe and pose no risks.

In addition to the OSH framework directive, there are other directives and regulations that expand on specific areas of safety and health. Further details on safety and health can be found at https://osha.europa.eu/en.

Directives are incorporated into domestic legislation by Member States and, as has been said above, they can introduce higher safety and health standards. It is important for each employee to be aware of the laws in the Member State where they are working.

14.3. Safety and health information

The European Agency for Safety and Health at Work (EU-OSHA) was set up to make European workplaces safer, healthier and more productive. It promotes a culture of risk prevention with the aim of improving working conditions in Europe.

EU-OSHA's work involves collecting, analysing and disseminating safety and health information throughout Europe; it also provides evidence to support the development of new policies. The agency website provides in-formation on a variety of safety and health topics (https://osha.europa.eu/en).

14.4. General safety in a laboratory animal facility

The framework directive places an obligation on all workers to cooperate with their employers to ensure that the working environment and working conditions are safe and pose no risks. All rules must be obeyed; individuals must not do anything that would risk their own or their colleagues' safety. If a situation is noticed that could develop into a risk to safety, it should be reported to a person in authority immediately. All accidents, no matter how trivial, must be reported and recorded, as should near misses.

A risk analysis should be carried out before any new programme of work commences. Staff working on the programme should be informed of the risks and have received appropriate training before beginning work. When conducting a risk assessment, the following stages should be completed:

— collect the relevant information;

— identify the possible hazards;

— assess the risks arising from the hazards;

— plan actions to eliminate or reduce risks;

— document the risk assessment.

(A hazard is the potential of a substance to cause harm. A risk is the likelihood that the substance will cause harm given the way it is intended to be used.)

14.4.1. Safe work practice

Working with live animals, biological agents, chemicals and laboratory equipment carries risks. If things go wrong while someone is working alone, these risks are made more serious because no one is available to give or get help. The greater the risks involved in any procedure, the more important it is not to work alone.

Appropriate Personal Protective Equipment (PPE) must be worn when working within an animal unit. What PPE is appropriate will depend on the work being carried out, but could include scrubs, gloves, head covering, laboratory coats, respiratory protection and safety footwear. Some animals, such as dogs and pigs, can produce noise at intensities that will, over time, damage human hearing. When working with these animals, some form of ear protection should be worn.

Eating and drinking is allowed only in designated areas. As protective clothing may be contaminated with Laboratory Animal Allergens (see **Chapter 14.4.2**), it should be changed or covered before entering the rest area. Regular handwashing should be carried out, even if gloves are worn.

14.4.2. Safety directly associated with animals

There are always risks when handling animals: they may bite or scratch and, if they are large animals, they may cause damage by kicking or even standing on a handler's foot. The likelihood of these things happening is greatly reduced with good handling technique (see **Chapter 13**). The seriousness of the injury will depend on the size of the animal and the type of experiment they are being used for. Every injury, no matter how minor, should be reported. These reports are essential to identify areas where safety improvements may need to be made. If, for instance, reports indicate that there is an increase in the number of rat bites that a unit is experiencing, there may be a need for more staff training, or it may indicate that a more aggressive strain of rat is being used and the handling techniques may need to be adapted accordingly.

Allergy to laboratory animals

An allergen is a substance that stimulates a specific type of immune response in sensitive humans. Exposure to urine, hair, feathers and dander of laboratory animals has the potential to cause an allergic response in approximately 25% of people in laboratory animal facilities. Rats and mice are the most common sources, but allergic responses are caused by other animals.

Susceptible individuals become sensitised within the first three years of working in an animal facility. Those most prone to developing an allergic response will have a personal or family history of allergies (Acton and McCauley, 2007). The symptoms are often mild to begin with, but they can develop into life-threatening conditions such as asthma. The severity of the reaction increases with the frequency of the exposure and the amount of the allergen present. Symptoms are summarised in **Table 14.1**.

Table 14.1. Symptoms of laboratory animal allergy

Location	Symptoms
Eyes	Itching, watering, red colour, swollen eyelids
Nose	Itching, running, sneezing
Skin	Itching, cracks, blisters, rash
Respiratory system	Coughing, wheezing, tightness in the chest, shortness of breath

Source: Stephen Barnett

In extreme cases an individual can suffer anaphylaxis, a very severe reaction in which, in addition to the symptoms mentioned in **Table 14.1**, blood pressure drops and the airways constrict, causing respiratory distress and asthma attacks. Medical assistance must be summoned immediately if this occurs. Individuals who have severe allergy usually carry a dose of adrenaline that relieves the symptoms. Even if the symptoms are relieved, medical assistance should still be sought.

Because exposure to LAAs is potentially very dangerous, people working with laboratory animals are required to undergo regular health checks so that symptoms can be picked up early. If any of the symptoms mentioned above are noticed, they should be reported to a supervisor or to the occupational health department so that the problem can be identified and action can be taken to avoid recurrence.

Measures can be taken to reduce exposure to allergens. These include:

- housing animals behind barriers such as IVCs or isolators;
- changes in working practices to reduce the emissions;
- good room ventilation systems;
- using filtered rodent cage change stations;
- moving animals within filtered containers;
- using appropriate PPE, including face masks or ventilated airflow helmets.

The use of PPE is seen as the last resort after all other protection mechanisms have been assessed; however, PPE is extremely effective when worn correctly and used in conjunction with other control measures, including those listed above.

Animal allergens can settle onto work clothes, equipment and other articles. To prevent contamination of people and the environment outside the animal facility, all PPE must be removed, hands washed thoroughly and equipment and other articles wiped down to remove the allergens before leaving the facility.

14.5. Biological hazards

The biological hazards that exist in an animal facility will depend on the type of work being carried out; for instance, studies involving animals infected with pathogens or radioactive materials will be more hazardous than studies involving non-infected animals. The risks associated with the animals also cover the risks around waste, tissues (including fur), carcasses, etc. arising from their use.

Animals infected with pathogens that may be hazardous to the health of workers have to be housed in containment accommodation appropriate to the level of risk they present (see **Chapter 4.3.6**). Staff must be appropriately trained to work in bio-containment units, provided with appropriate PPE and offered vaccinations if they are available.

14.5.1. Zoonoses

Zoonoses are diseases that can be contracted by humans from animals; they are therefore a risk to people working with animals. Animals bred and used in SPF barrier facilities are unlikely to be infected with a zoonosis, but people working with farm animals or non-human primates may be at risk. There are some 200 zoonoses of importance, and the list is growing. They include the bacterial disease salmonellosis, the fungal disease ringworm, the parasite-caused infection toxocariasis and the viral disease rabies (WHO, 2020).

14.5.2. Disposal of biological waste

All biological waste must be disposed of in accordance with local rules. The method used will depend on the potential risk from the waste. All infectious material, including animal waste, will usually be autoclaved out of the unit and either taken to an on-site incinerator or removed by a specialist clinical waste company. Non-infectious animal waste will not need to be autoclaved before disposal. The method of disposal of waste from a radiation suite will depend on the type of radiation used and its strength. It is imperative to follow instructions from senior colleagues when handling radioactive material.

Several people may have to handle biological waste material before it reaches its final destination. Waste must be packaged so that subsequent handlers are protected from direct contact with the waste. Double-bagging in clearly labelled, heavy-duty plastic bags, taking care not to overfill the bags, is one suitable method. The bags should be autoclaved or disinfected before leaving the unit.

Many sharp instruments are used in biological work (e.g. scalpels, hypodermic needles). Glass containers or vials are often used, which can be easily broken, giving rise to sharp pieces of glass. The risk of wounding is high and, if the item is contaminated, harmful materials may be introduced directly into the bloodstream. When disposing of items contaminated with biological materials, it is necessary to sterilise or disinfect them to eliminate any potentially hazardous material. Sharp items (or glass containers that could easily break) must be kept separate from other waste. They should be placed in dedicated hard plastic containers, for example a 'sharps' bin, to prevent them from causing danger to any subsequent handlers (**Figure 14.1**).

All accidents involving work with biological agents must be reported immediately and expert advice sought.

Figure 14.1. Sharps bin

Source: Stephen Barnett

14.6. Manual handling

Musculoskeletal disorders (MSDs) are the most common work-related health problem reported in the EU (EU-OSHA, 2024). There are several causes of MSD, but approximately 34% of all cases are reported to be caused by manual handling. 25% of European workers suffer from back pain, so it has great human welfare and financial importance. An MSD is rarely caused by one event; it is often the result of a build-up of small incidents over a long period. It is not caused only by lifting heavy loads; a small load lifted incorrectly can also lead to MSDs. In addition to lifting, manual handling includes holding, putting down, pushing, pulling and carrying a load. Animal care staff are taught how to handle laboratory animals, but there can be a risk of MSD even if small animals are handled awkwardly or if larger animals struggle when being carried.

MSD covers damage to muscles, tendons, ligaments, bones and related structures, but other injuries, such as cuts and bruises, can also occur.

Before any load is handled manually, a risk analysis must be carried out. The first question to be considered is if the load needs to be manually handled at all. For instance, perhaps a mechanical aid such as a lifting platform or fork lift truck could be used. If manual handling is the only option, it should be done by an employee who is trained in the correct method.

When assessing the risk associated with handling a particular load, the load itself must be considered. The weight and size must be assessed; loads that can be held close to the body are less likely to cause damage. It is also necessary to assess its stability; for example, is there any possibility that items within the load will move and unbalance the load while it is being lifted or carried?

The environment through which the load must be moved must be considered.

— Is there enough space to move the load?

— Is the floor on one level? Is it slippery or uneven?

— Are there obstacles in the way, including doors?

— Is there enough light to see what has to be done?

The staff who will do the lifting must be considered: their strength and fitness must be assessed, including any history of back problems.

EU-OSHA has produced several factsheets on manual handling. E-facts 14 ('Hazards and risks associated with manual handling in the workplace') and 44 ('Checklist for the prevention of manual handling risks') provide more information on the subject (EU-OSHA, 2007, 2008). They are available, free of charge, from http://osha.europa.eu.

14.6.1. Repetitive strain injuries

Repetitive strain injuries are another form of MSD. They are caused by prolonged repetition of a particular manual task, for example rodent cage changing or bottle filling, especially if it requires using force or holding the body in an awkward position. Common signs are numbness, tenderness, pain and pale cold skin in the affected area. Such injuries can be avoided by limiting repetitious tasks as much as possible, alternating hands, reducing the amount of force used and paying attention to body position. It is important to take regular breaks, move position, stretch and allow time for the body to recover.

14.7. Hazard warning signs

Signs play an important part in warning people of the hazards associated with a process, substance or work area. Directive 92/58/EEC establishes the minimum requirements for the provision of safety and/or health signs at work. Member States have the freedom to maintain or adopt more stringent requirements if they wish.

There is an internationally agreed set of hazard warning signs, produced by the International Organization for Standardization (ISO). These signs can be used in the EU but, if there is a difference between the ISO and Directive 92/58/EEC, the directive must be followed. The European Commission has published non-binding guidelines regarding Directive 92/58/EEC: safety and/or health signs at work (ACHSW, 2020).

The warning signs are divided into mandatory, prohibition, warning, emergency and firefighting. **Figure 14.2** shows examples of these signs. (The examples shown comply with both Directive 92/58/EEC and ISO.)

Figure 14.2. The colour codes of the major types of hazard warning signs

Sign	Description	Examples
Mandatory These indicate what protective equipment must be worn to prevent injury.	Blue and white	
Prohibition These prohibit actions that could endanger life or cause injury.	Red circle with red diagonal line	
Warning These warn of specific hazards that are present.	Yellow triangles	
Emergency These indicate identification and location of escape routes, first aid and safety equipment.	Green and white	
Fire Fighting These indicate location of fire fighting equipment and storage of flammable substances.	Red	

Source: Stephen Barnett

14.8. Chemical hazards

European Regulation (EC) No. 1907/2006 established the European Chemicals Agency (ECHA) and REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) with the aim of protecting human health and the environment from risks posed by chemicals. The regulations also promotes alternative methods for the hazard assessment of chemicals in order to reduce the use of animals.

All chemical companies must register their products with the ECHA, together with information on the risks and how they can be managed. The ECHA checks the information given by companies and can, if it thinks it appropriate, restrict the use of those it believes to be too dangerous.

The ECHA has developed a database on chemicals, which can be consulted through its website (http://echa.europa.eu/regulations/reach/legislation).

All chemicals must be handled with care and in accordance with the instructions. Even those that are apparently harmless could react violently when they come into contact with other chemicals.

The following list refers to some of the ways in which chemicals can be harmful:

- carcinogenic – affecting cell division, resulting in overgrowth of tissue, causing tumours (e.g. many tarry substances, blue asbestos);
- corrosive – producing burns and other damage to tissues (e.g. strong acids and alkalis);
- explosive – releasing intense heat and large amounts of gas when exposed to a suitable stimulus, such as a spark (e.g. nitroglycerine, flour);
- flammable – catching fire easily because it has a low flash point (e.g. ether and many other solvents);
- irritant – causing local tissue reaction to its presence (e.g. dust);
- toxic – causing poisoning (e.g. mercury, strychnine, organophosphorus compounds);
- teratogenic – causing the malformation of developing embryos/fetuses (e.g. thalidomide);
- mutagenic – causing an increased occurrence of mutations in the populations of cells and/or organisms;
- genotoxic – causing DNA damage by interfering with normal cell replication processes.

Chemicals can enter the body by the same routes as pathogenic organisms, that is, by ingestion, inhalation and skin absorption. In all of these cases, damage may be local or the chemical may be absorbed and distributed around the body, causing a systemic effect.

The amount of damage done to tissues is affected by the concentration of the chemical, the exposure time and the frequency of exposure.

14.8.1. Safe use of chemicals

Before chemicals can be used in a work situation, a risk assessment must be carried out and measures established to ensure that they are used in a controlled and safe manner. Manufacturers and suppliers of chemicals must communicate the hazards associated with their chemicals through pictograms attached to their containers, safety data sheets and standard safety sheets (as required by the EU

Classification, Labelling and Packaging Regulations of 2015 (revised 2024). Examples of the pictograms are in **Figure 14.3**. Once the hazards are identified, means of controlling them must be developed. This will result in an SOP or safe work practice instructions, which will detail how the chemical must be used in the workplace. Included in the instructions will be appropriate protective clothing to be worn and action to be taken in the event of accidents and spillage.

Staff using any chemical must be provided with adequate information, be trained on to use it and understand that it should be used only in the correct manner, for example with the correct dilution of disinfectant. A chemical used in an appropriate manner should have little or no risk attached to it, but if misused or mishandled it can be very dangerous.

All safety measures introduced must be monitored on a regular basis to ensure that they are still effective; this is especially true for the use of PPE.

Figure 14.3. Hazard pictograms

Source: Stephen Barnett

14.8.2. Spillage of chemicals

Before a chemical is used, the hazards associated with it and the action to be taken in the event of a spillage must be known. The basic principle for dealing with a chemical spillage is containment and using an appropriate method to clear and safely dispose of the chemical. This often means soaking up the liquid in an inert absorbent material and clearing it into a suitable container for safe disposal (note, sawdust or other wood products should not be used for this purpose). To ensure that an inert material is used, specialised spillage kits should be available.

The control, supply and use of controlled drugs, prescription-only medicines and other therapeutic substances used on animals in authorised establishments are the responsibility of the Designated Veterinarian.

14.8.3. Transporting chemicals

Gas cylinders should be moved in an upright position in a specialised transport trolley to ensure that the cylinder valve is not damaged. Winchester bottles must be transported within a dedicated Winchester holder, not carried by the neck (**Figure 14.4**).

Figure 14.4. Winchester carriers

Source: Stephen Barnett

Legislation exists to regulate the disposal of chemicals in order to prevent environmental pollution. Highly toxic materials such as solvents must not enter sewage systems. Specialist chemical disposal companies are available to remove hazardous waste.

14.9. Fire

Fire begins when three elements come together:

— oxygen (usually in air, but it can be supplied by oxidising chemicals);

— sufficient heat to provide ignition;

— a fuel.

If one of these elements is absent, there will be no fire. Methods of extinguishing fires work by removing one of the three elements, usually reducing heat or excluding oxygen.

Fires are divided into classes based on the fuel present and the way in which the fire can be extinguished. The classes are:

— class A fires – carbonaceous material that forms glowing embers (e.g. paper, wood, coal, cloth, plastics);

— class B fires – flammable liquids (e.g. alcohol, acetone);

— class C fires – flammable gases;

— class D fires – flammable metals (e.g. magnesium, aluminium, sodium, potassium);

— electrical fires.

Table 14.2 shows a range of fire extinguishers and examples of the types of fire for which they are appropriate. It is essential to select the appropriate extinguisher, as using the wrong one can increase the danger to the person using it and may make the situation worse. All new extinguishers must be of the EN3 type. The overall colour of each extinguisher is red, and there is a panel on the front that indicates what type of extinguisher it is. The label also has wording and pictograms to show what fire types the extinguisher is suitable for fighting. The label must be checked to ensure that the correct extinguisher is being used (**Figure 14.5**).

Figure 14.5. A carbon dioxide (CO^2) fire extinguisher showing the black panel and instructions for use

Source: Stephen Barnett

Table 14.2. Examples of Fire extinguishers and their uses

Class of fire	Type of Extinguisher (Colour of label)			
	Foam (cream)	ABC powder (blue)	Carbon dioxide (black)	Water (red)
A	Yes	Yes	No	Yes
B	Yes	Yes	Yes	No
C	No	Yes	No	No
D	No	Special dry powder	No	No
Electrical	No	Yes	Yes	No

Source: Stephen Barnett

The correct extinguisher must be used, and it must be used in the correct way, so all staff should undergo firefighting training. Staff should always follow the instructions of local fire officers on the most appropriate way to react to a fire.

Any door will stop smoke, and even hold back the fire for a while. Closed doors restrict the supply of air, which reduces the intensity of the fire. Doors designated as fire doors are specifically designed to retard the progress of both smoke and fire, and therefore must be kept closed when not in use. Some fire doors include a strip of material that expands when heated to seal the gap around the door. Some are kept open by magnetic catches that are automatically released once the alarm is raised.

It is essential to know the positions of fire alarm buttons in any work area so that they may be used quickly in an emergency. The traditional alarm bell should not be used in animal units, particularly those containing small rodents, as the noise of a clanging bell distresses the animals. Instead, some units are fitted with flashing-light warning systems in every room, or with alarms that sound at a decibel and frequency readily heard by a human, but outside the hearing range of animals, so they do not upset them. Some alarm systems have a two stage warning system, the first gives an intermittent ring or a flashing light which is the signal to get ready to leave. When the signal becomes a continuous one staff should leave immediately.

Staff must familiarise themselves with the nearest fire exit and the designated assembly points outside the building. Fire exits must be kept free from obstruction and be properly signposted.

If a fire is discovered, it is imperative that the alarm is raised immediately, even if it seems easy to control. Any delay in raising the alarm could have serious consequences if the fire gets out of hand.

When the fire alarm is heard, it must be assumed that there is a fire and the building must be evacuated. Local evacuation procedures must be followed. If there is time, any apparatus that could cause extra problems should be switched off and all doors (and windows if there are any) should be closed.

Staff should proceed to the designated assembly point without delay so that they can be accounted for. Staff should not attempt to retrieve personal belongings, human life must come first. In most cases, it is not possible to evacuate animals.

14.10. Electrical hazards

Electricity is a form of energy that can be used to provide heat, light, sound, movement, etc. It consists of a flow of electrons through a conducting material (e.g. a wire or cable). The amount of flow is called the current and is measured in units called amperes (or amps, symbol A). The pressure at which

the current is drawn through the conducting material is called the voltage (volts, symbol V). In the EU the standard electricity supplies are 220–240 volts. The power consumption of electrical apparatus is measured in units called watts (symbol W); power is current multiplied by voltage, watts = amps × volts (W = A × V).

The amount of current that flows through a cable depends on the amount of power being used by the appliance it is connected to. Information on power consumption and current requirement should be available on the appliance itself, or it can be calculated from the formula:

current (amps, A) = (power (watts,W))/(voltage (volts,V))

In order for a current to flow, there must be a complete circuit. Normally, electrons flow from the source through a connecting wire (the 'live' wire) to the appliance, and then back from the appliance through the 'neutral' wire to the source. If there is a break in the wire or a fault in the appliance, the current will not flow. However, if the opportunity arises, the current will flow through any other conductor; if a human touches a live wire or appliance, the current will flow through the human, with disastrous results.

Electrical accidents can result in:

- electric shock, when electricity flows through nerves, organs and muscles, producing adverse reactions (e.g. a heart attack);
- electrical burns, from the heating effect of the current on tissue;
- electrical fires, caused by overheating or arcing of the equipment when in contact with a fuel (e.g. paper).

Maintenance checks of portable appliances should be carried out on a regular basis, to ensure that they are properly insulated and performing in a safe manner.

All rigid and flexible cables, plug tops, sockets and adapters are rated with the maximum current that it is safe to draw through them. When a higher current is drawn, there is a danger of overheating, which can cause the insulation material to melt, increasing the risk of electrical shock and fire. Coiled extension leads usually have two current ratings stated on them: one for when they are fully extended and a lower one for when they are left partially wound. This is necessary because heat cannot dissipate efficiently if they are coiled. Sometimes it is necessary to use adapters to connect more than one plug to a socket or to use an extension lead with a distribution board with several outlets in order to accommodate new or temporary equipment. Where these are used, the total amount of current drawn by all of the appliances must not exceed the rated value of the cable and, whatever the rating of the cable, must not exceed the rating of the plug, because that is the maximum that standard wall plugs are designed to carry. Extension cables must not be used as a permanent power supply.

There is an increased risk when using electrical appliances in damp or wet conditions, because the moisture conducts electricity over the surface of the insulating material.

To minimise the risk, electrical sockets in potentially damp areas should be fitted with waterproof covers. All electrical cables must be kept clear of small formations of puddles, and a residual current device or circuit breaker must be fitted, which will cut off the power source in the event of a problem. Care must be taken to prevent trailing electrical leads from creating a trip hazard or becoming damaged by the wheels of cage racks or trolleys, and to prevent leads from trailing over animal cages or pens where they can be chewed.

14.11. Accident procedures

If an accident involving trauma or unconsciousness to either a human or an animal is discovered, it is important not to make things any worse! The situation should be assessed before any action is taken. If it is possible to do so without causing danger to oneself or further danger to the injured party, the primary cause should be removed; for example, in the case of an electric shock, the power supply should be turned off. Persons who are not qualified in first aid should then summon assistance from someone better qualified to deal with the situation.

The role of the qualified first-aider is to prevent the condition from deteriorating, to preserve life and to promote recovery.

Reporting accidents and ill health at work is a legal requirement. The information can be used both at the local level and by the enforcing authorities to see where injuries, ill health and accidents are occurring, and to advise on preventive action.

References

ACHSW (2020)

European Commission, Directorate-General for Employment, Social Affairs and Inclusion, (2022) Non-binding guidelines regarding Directive 92/58/EEC: safety and/or health signs at work. Publications Office of the European Union. https://data.europa.eu/doi/10.2767/686887

Acton, D. and McCauley, L. (2007), 'Laboratory animal allergy: an occupational hazard', *AAOHN Journal*, Vol. 55, No 6, pp. 241–244, https://doi.org/10.1177/216507990705500604.

Council Directive 89/391/EEC of 12 June 1989 on the introduction of measures to encourage improvements in the safety and health of workers at work, OJ L 183, 29.6.1989, p. 1, ELI: https://eur-lex.europa.eu/eli/dir/1989/391/oj.

ECHA, 'REACH legislation', ECHA website, http://echa.europa.eu/regulations/reach/legislation.

EU-OSHA website, https://osha.europa.eu/en.

EU-OSHA (2007), 'E-fact 14: Hazards and risks associated with manual handling in the workplace', EU-OSHA website, https://osha.europa.eu/en/publications/e-fact-14-hazards-and-risks-associated-manual-handling-workplace.

EU-OSHA (2008), 'E-fact 44: Checklist for the prevention of manual handling risks', EU-OSHA website, https://osha.europa.eu/en/publications/e-fact-44-checklist-prevention-manual-handling-risks.

EU-OSHA (2024) Prevention strategies for MSD in the health care sector. https://oshwiki.osha.europa.eu/en/themes/prevention-strategies-msds-healthcare-sector.

European Agency for Safety and Health at Work (2017), https://op.europa.eu/en/publication-detail/-/publication/07e5430e-66ad-11e7-b2f2-01aa75ed71a1/language-en.

WHO (World Health Organization) (2020), 'Zoonoses', WHO website, https://www.who.int/news-room/fact-sheets/detail/zoonoses.

Further reading

EU-OSHA WIKI website, https://oshwiki.osha.europa.eu/en.

Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals. http://data.europa.eu/eli/reg/2006/1907/oj.

Contribution of this chapter to the Three Rs.

Most of this book is concerned with the welfare of animals; this chapter is different, as it is about the safety and health of the people who work in animal facilities. However, in many cases, actions that minimise risk to the safety and health of humans will also protect animals. For example, protective clothing protects wearers and animals, and using chemicals safely protects both animals and staff. When designing safety features, the welfare of animals should be considered where possible; for example, use fire alarms that operate outside the hearing range of the animals.

Self-assessment questions

1. What three obligations are placed on workers by Directive 89/391/EEC (the occupational safety and health framework directive)?
2. Before a load is handled manually, a risk analysis should be undertaken. What should be considered in that analysis?
3. What action should be taken if a fire alarm sounds?
4. What do the following hazard warning signs indicate?
    (a) Green and white.
    (b) Red circle with red diagonal line.
    (c) Blue and white.
5. Where can information on the hazards associated with specific chemicals be found?

15. Correlation of the Manual with EU Education and Training Framework[17] Modules and Learning Outcomes

Instruction for tutors: this book has been written to support new employees starting their careers as animal caretakers/technicians in laboratory animal facilities. It covers many of the Learning Outcomes stated in the EU Education and Training document but does not cover them all. On its own, it will not provide all of the information required to become a qualified animal caretaker/technician but it will provide a good understanding of how laboratory animal facilities function and the important role caretakers and technicians have within them. In addition to the material in this book, students will need to learn from other sources, from tutors and from experienced colleagues in the workplace.

Table 15.1. Mapping Manual Chapters to Modules and Learning Outcomes

Chapter	Modules and Learning Outcomes (LO)
1	Module 1: [18]
2	Module 12: all LOs (12.1 – 12.24)
3	Module 2: LOs 2.1, 2.2, 2.4, 2.5, 2.6, 2.7, 2.11, 2.12, 2.13 Module 3: LO 3.1.3
4	Module 3: 3.1.9 Module 4: LO 4.5, 4.9 Module 5: LO 5.1, 5.2, 5.3
5	Module 3: 3.1.2, 3.1.4, 3.1.7 Module 4: LOs 4.2, 4.3
6	Module 3: LO 3.1.9 Module 4: LO 4.1
7	Module 3: LO 3.1.5 Module 4: LO 4.6
8	Module 3: LO 3.1.1, 3.1.7, 3.1.9 Module 4: LO 4.10
9	Module 4: LO 4.11
10	Module 4: LO 4.8
11	Module 4: LO 4.4 Module 7: LOs 7.1, 7.2, 7.3, 7.4, 7.5,7.9
12	Module 6: LOs 6.1.1, 6.1.2
13	Module 4: LO 4.4, LO 4.7
14	Module 4: LO 4.13

Source: Authors' and editors' own elaboration

[17] https://environment.ec.europa.eu/topics/chemicals/animals-science_en#implementation – see section Guidance documents

[18] **Chapter 1** gives a general introduction to the requirements of the legislation and includes information on all the LOs, but doesn't explicitly cover the circumstances under which animals should be removed from study (LO 1.11). Neither does it explain how the Directive has been implemented within various National Legislations.

Table 15.2. Mapping Modules and Learning Outcomes to Manual Chapters

Module	Chapter	Learning outcomes (LO)
1	1	[19]
2	3	LOs 2.1, 2.2, 2.4, 2.5, 2.6, 2.11, 2.12, 2.13
3	3	LO 3.1.3
	4,6	LO 3.1.9
	5	LOs 3.12, 3.14, 3.17
	7	LO 3.1.5
	8	LOs 3.1.1, 3.1.7, 3.1.9
4	6	LO 4.1
	5	LOs 4.2, 4.3
	4	LOs 4.5, 4.9
	11	LOs 4.4, 4.12
	7	LO 4.6
	13	LO 4.7
	10	LO 4.8
	8	LO 4.10
	9	LO 4.11
	14	LO 4.13
5	4	LOs 5.1, 5.2, 5.3
6	12	LOs 6.1.1, 6.1.2
7	11	LOs 7.1, 7.2, 7.3, 7.4, 7.5, 7.9
	3	LOs 7.7, 7.8
12	2	All LOs: 12.1-12.24

Source: Authors' and editors' own elaboration

[19] **Chapter 1** gives a general introduction to the requirements of the legislation and includes information on all the LOs, but doesn't explicitly cover the circumstances under which animals should be removed from study (LO 1.11). Neither does it explain how the Directive has been implemented within various National Legislations.

References and further reading

ACHSW (2020)

Acton, D. and McCauley, L. (2007), 'Laboratory animal allergy: an occupational hazard', *AAOHN Journal*, Vol. 55, No 6, pp. 241–244, https://doi.org/10.1177/216507990705500604.

Animal Technology and Welfare, https://www.atwjournal.com/.

AVMA (American Veterinary Medical Association) (2020), 'Guidelines for the euthanasia of animals: 2020 edition', AVMA website, https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf.

Barnett, S. W. (2007), 'The supply and production of protected animals', in: Barnett, S. W. (ed.), Manual of Laboratory Animal Technology, pp 1-12. Blackwell Publishing, Oxford.

Baumans, V. (2005), 'Science-based assessment of animal welfare: Laboratory animals', *Revue scientifique et technique*, Vol. 24, No 2, pp. 503–513.

BioTechniques (2023), 'What is organ-on-a-chip technology?', YouTube website, https://www.youtube.com/watch?v=8tlHd5pYH0Y.

Blackburn, S. (2016), Oxford Dictionary of Philosophy, 3rd edition, Oxford University Press, Oxford.

Brambell, R. (1965), 'Report of the technical committee to enquire into the welfare of animals kept under intensive livestock husbandry systems', HM Stationery Office, London, pp. 1–84.

Burn, C. C., Mazlan, N. H. B., Chancellor, N. and Wells, D. J. (2021), 'The pen is milder than the blade: Identification marking mice using ink on the tail appears more humane than ear-punching even with local anaesthetic', *Animals*, Vol. 11, No 6, p. 1664, http://doi.org/10.3390/ani11061664.

Byers, S. L., Wiles, M. V., Dunn, S. L. and Taft, R. A. (2012), 'Mouse oestrous cycle identification tool and images', *PLOS One*, Vol. 7, No 4, e35538, https://doi.org/10.1371/journal.pone.0035538.

Clemence M & Leaman J (2016). Public attitudes to animal research in the UL 2016. Ipsos Mori. https://www.ipsos.com/sites/default/files/publication/1970-01/sri-public-attitudes-to-animal-research-2016.pdf.

Clough, G. (2007), 'The environment', in: Barnett, S. W. (ed.), Manual of Animal Technology, Blackwell Publishing, Oxford.

Cloutier, S., LaFollette, M. R., Gaskill, B. N., Panksepp, J. and Newberry, R. C. (2018), 'Tickling, a technique for inducing positive affect when handling rats', *Journal of Visualized Experiments*, Vol. 135, e57190, https://doi.org/10.3791/57190.

Commission Recommendation 2007/526/EC of 18 June 2007 on guidelines for the accommodation and care of animals used for experimental and other scientific purposes (notified under document number C(2007) 2525), OJ L 197, 30.7.2007, p. 1, ELI: https://eur-lex.europa.eu/eli/reco/2007/526/oj.

Council Directive 89/391/EEC of 12 June 1989 on the introduction of measures to encourage improvements in the safety and health of workers at work, OJ L 183, 29.6.1989, p. 1, ELI: https://eur-lex.europa.eu/eli/dir/1989/391/oj.

Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing, OJ L 303, 18.11.2009, p. 1, ELI: http://data.europa.eu/eli/reg/2009/1099/oj.

Council Regulation (EC) No 1/2005 on the protection of animals during transport and related operations. https://eur-lex.europa.eu/eli/reg/2005/1/oj.

Council regulation (EC) No 33/97 on the protection of wild fauna and flora by regulating the trade therein http://data.europa.eu/eli/reg/1997/338/oj.

Dahlborn, K., Bugnon, P., Nevalainen, T., Raspa, M., Verbost, P., et al. (2013), 'Report of the Federation of European Laboratory Animal Science Associations Working Group on animal identification', *Laboratory Animals*, Vol. 47, No 1, pp. 2–11, https://doi.org/10.1177/002367712473290.

Damon, E. G., Eidson, A. F., Hobbs, C. H. and Hahn, F. F. (1986), 'Effect of acclimatisation on nephrotoxic response of rats to uranium', Laboratory Animal Science, Vol. 36, No 1, pp. 24–27.

Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC), OJ L 262, 17.10.2000, p. 21, ELI: http://data.europa.eu/eli/dir/2000/54/oj.

Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC – Commission Declaration, OJ L 106, 17.4.2001, p. 1, ELI: https://eur-lex.europa.eu/eli/dir/2001/18/oj.

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, OJ L 276, 20.10.2010, p. 33, ELI: https://eur-lex.europa.eu/eli/dir/2010/63/oj/eng.

Dolan, K. (1999), Ethics, Animals and Science, Blackwell Science, Oxford.

Dolan, K. (2007), 'The ethical implications of the use of animals in scientific procedures', in: Barnett, S. W. (ed.), Manual of Animal Technology, pp. 326-347 Blackwell Publishing, Oxford.

ECHA, 'REACH legislation', ECHA website, http://echa.europa.eu/regulations/reach/legislation.

Education and Training Platform for Laboratory Animal Science (ETPLAS) website, https://etplas.eu/en.

EU-OSHA (2024) Prevention strategies for MSD in the health care sector. https://oshwiki.osha.europa.eu/en/themes/prevention-strategies-msds-healthcare-sector.

EU, 'EUR-Lex: Access to European Union law', EUR-Lex website, https://eur-lex.europa.eu/.

EU-OSHA (2007), 'E-fact 14: Hazards and risks associated with manual handling in the workplace', EU-OSHA website, https://osha.europa.eu/en/publications/e-fact-14-hazards-and-risks-associated-manual-handling-workplace.

EU-OSHA (2008), 'E-fact 44: Checklist for the prevention of manual handling risks', EU-OSHA website, https://osha.europa.eu/en/publications/e-fact-44-checklist-prevention-manual-handling-risks.

EU-OSHA website, https://osha.europa.eu/en.

EU-OSHA WIKI website, https://oshwiki.osha.europa.eu/en.

European Agency for Safety and Health at Work (2017), https://op.europa.eu/en/publication-detail/-/publication/07e5430e-66ad-11e7-b2f2-01aa75ed71a1/language-en.

European Commission (2010), 'Science and technology report – Special Eurobarometer 340', SP340, https://europa.eu/eurobarometer/surveys/detail/806.

European Commission (2023), Commission staff working document – Summary report on the statistics on the use of animals for scientific purposes in the Member States of the European Union and Norway in 2020, SWD(2023) 84 final, Brussels.

European Commission, 'ALURES (Animal use reporting – EU system) – EU statistical database on the use of animals for scientific purposes under Directive 2010/63/EU', Section 1: Numbers and origins of animals in research and testing https://webgate.ec.europa.eu/envdataportal/content/alures/section1_number-of-animals.html.

European Commission, 'ALURES (Animal use reporting – EU system) – EU statistical database on the use of animals for scientific purposes under Directive 2010/63/EU', Section 2: Uses in research and testing https://webgate.ec.europa.eu/envdataportal/content/alures/section2_number-of-uses.html.

European Commission, 'Animals in science – Implementation', European Commission website, https://environment.ec.europa.eu/topics/chemicals/animals-science_en#implementation.

European Commission, 'Animals in science – The "Three Rs"', European Commission website, https://environment.ec.europa.eu/topics/chemicals/animals-science_en#the-three-rs.

European Commission, Directorate-General for Employment, Social Affairs and Inclusion, (2022) Non-binding guidelines regarding Directive 92/58/EEC: safety and/or health signs at work. Publications Office of the European Union. https://data.europa.eu/doi/10.2767/686887.

European Commission: Directorate-General for Environment, Framework for the genetically altered animals under Directive 2010/63/EU on the protection of animals used for scientific purposes, Publications Office of the European Union, 2022, https://data.europa.eu/doi/10.2779/499108.

Federation of European Laboratory Animal Science Associations (FELASA) website, https://felasa.eu/.

Festing, M. F. W. (1994), 'Reduction of animal use: Experimental design and quality of experiments', *Laboratory Animals*, Vol. 28, pp. 212–221.

Festing, M.F.W. and Lutz C. (2010), 'Introduction to laboratory animal genetics', in: Hubrect, R and Kirkwood, J. (eds), The UFAW handbook on the care and management of laboratory and other research animals, 8th edition, pp 37-60. Wiley-Blackwell.

Flecknell, P. (1996), Laboratory Animal Anaesthesia – A practical introduction for research workers and technicians, 2nd edition, Academic Press, San Diego, CA.

Francis, R. (2010), 'Refinement in in-house animal production and breeding', in: Hubrect, R. and Kirkwood, J. (eds), The UFAW handbook on the care and management of laboratory and other research animals, 8th edition, pp 163-168. Wiley-Blackwell.

Gerson, P. (2007), 'The isolation of laboratory animals that are a potential human risk', in: Barnett, S. W. (ed.), Manual of Animal Technology, Blackwell Publishing, Oxford.

Guillén, J., Prins, J-B., Smith, D., Degryse, A.-D. (2014), 'The European framework on research animal welfare regulations and guidelines', in: Guillén, J. (ed.), *Laboratory Animals*: Regulations and recommendations for global collaborative research, Academic Press, San Diego, CA, pp. 117–188.

Harrison, R. (1964), Animal Machines – The new factory farming industry, Vincent Stuart Publishers.

Hawkins, P., Morton, D. B., Burman, O., Dennison, N., Honess, P., et al. (2011), 'A guide to defining and implementing protocols for the welfare assessment of laboratory animals: eleventh report of the

BVAAWF/FRAME/RSPCA/ UFAW Joint Working Group on Refinement', *Laboratory Animals*, Vol. 45, pp. 1–13.

Hawkins, P., Prescott, M. J., Carbone, L., Dennison, N., Johnson, C., et al. (2016), 'A good death? Report of the second Newcastle meeting on laboratory animal euthanasia', *Animals*, Vol. 6, No 9, p. 50, https://doi.org/10.3390/ani6090050.

Hurst, J. L. and West, R. S. (2010), 'Taming anxiety in laboratory mice', *Nature Methods*, Vol. 7, pp. 825–826, https://doi.org/10.1038/nmeth.1500.

IATA, 'Live animals', IATA website, https://www.iata.org/en/programs/cargo/live-animals/.

Ingraham, A. and Fleischer, T. M. (2003), 'Disinfectants in laboratory animal science: What are they and who says they work?', *LabAnimal*, Vol. 32, No 1, pp. 36–40.

Joint Research Centre, 'EU Reference Laboratory for alternatives to animal testing (EURL ECVAM)', European Commission website, https://joint-research-centre.ec.europa.eu/projects-and-activities/reference-and-measurement/european-union-reference-laboratories/eu-reference-laboratory-alternatives-animal-testing-eurl-ecvam_en.

Kemp, R. W. (2007), 'Animals with a defined microbiological status', in: Barnett, S. W. (ed.), Manual of Animal Technology, Blackwell Publishing, Oxford.

Kinally, A., Onions, L. and Glenn, S. (2023), 'Validating the use of box training as a refinement to rabbit breeding', Animal Technology and Welfare, Vol. 22, No 1, pp. 35–40.

Klabukov, I., Shestakova, V., Krasilnikova, O., Smirnova, A., Abramova, O., et al. (2023), 'Refinement of animal experiments: Replacing traumatic methods of laboratory animal marking with non-invasive alternatives', *Animals*, Vol. 13, No 22, p. 3452, https://doi.org/10.3390/ani13223452.

Krause, J., McDonnell, G. and Riedesel, H. (2001), 'Biodecontamination of animal rooms and heat-sensitive equipment with vaporized hydrogen peroxide', Contemporary Topics in Laboratory Animal Science, Vol. 40, No 6, pp. 18–21.

Larson, M. A. (ed.) (2020), Transgenic Mouse: Methods and protocols, Methods in Molecular Biology, Vol. 2066, Springer.

Latham, N. (2010), 'A brief introduction to welfare assessment: A "toolbox" of techniques', in: Hubrecht, R. and Kirkwood, J. (eds), The UFAW handbook on the care and management of laboratory and other research animals, 8th edition, Wiley-Blackwell, Chichester, pp. 76–91.

Laule, G. (2010), 'Positive reinforcement training for laboratory animals', in: Hubrecht, R. and Kirkwood, J. (eds), The UFAW handbook on the care and management of laboratory and other research animals, 8th edition, Wiley-Blackwell, Chichester, pp. 206–218.

Lowe, B. L. (2007), 'Nutrition', in: Barnett, S. W. (ed.), Manual of Animal Technology, pp.175-197. Blackwell Publishing, Oxford.

Low-Marchelli J (2017), 'Strategies to minimize genetic drift and Maximise experimental reproducibility in mouse research.' Charles River Laboratories International Inc. https://animalab.eu/knowledge-base/strategies-to-minimize-genetic-drift-and-maximize-experimental-reproducibility-in-mouse-research.

Meller, A., Kasanen, I., Ruksenas, O., Apanaviciene, N., Baturaite, Z., et al. (2011), 'Refining cage cleaning routines: Comparison of cardiovascular responses to three different ways of cage change in rats', Laboratory Animals, Vol. 45, No 3, pp. 167–173.

Meyer N, Gottschalk R, Jakobi L, Schonhoff A-M and Touma C (2025) Nature Scientific Reports https://www.nature.com/articles/s41598-025-07384-w.

Mongillo, P., Scandurra, A., Kramer, R. S. S. and Marinelli, L. (2017), 'Recognition of human faces by dogs (Canis familiaris) requires visibility of head contour', *Animal Cognition*, Vol. 20, pp. 881–890, https://doi.org/10.1007/s10071-017-1108-4.

NC3Rs (2018), 'Mouse handling: Tutorial', NC3Rs website, https://www.nc3rs.org.uk/mouse-handling-video-tutorial.

NC3Rs (2021), 'Environmental monitoring for rodent health surveillance', NC3Rs website, https://nc3rs.org.uk/events/environmental-monitoring-rodent-health-surveillance.

NC3Rs (2022), 'Blood sampling', NC3Rs website, https://nc3rs.org.uk/3rs-resources/blood-sampling.

NC3Rs (2022), 'Welfare assessment – Grimace scales', NC3Rs website, https://nc3rs.org.uk/3rs-resources/grimace-scales.

NC3Rs (2024), 'Colony management best practice', NC3Rs website, https://www.nc3rs.org.uk/3rs-resources/breeding-and-colony-management/colony-management-best-practice.

NC3Rs (2024), 'Minimising aggression in group-housed male mice', NC3Rs website, https://nc3rs.org.uk/3rs-resource-library/minimising-aggression-group-housed-male-mice.

NC3Rs, 'Evaluating environmental enrichment', NC3Rs website, https://nc3rs.org.uk/3rs-resource-library/evaluating-environmental-enrichment.

NC3Rs, 'Procedures with care', NC3Rs website, https://nc3rs.org.uk/3rs-resources/procedures-care.

Obernier, J. A. and Baldwin, R. L. (2006), 'Establishing an appropriate period of acclimatisation following transportation of laboratory animals', *ILAR Journal*, Vol. 47, No 4, pp. 364–369, https://doi.org/10.1093/ilar.47.4.364.

Pallares, P. and Gonzalez-Bulnes, A. (2009), 'Use of ultrasound imaging for early diagnosis of pregnancy and determination of litter size in the mouse', *Laboratory Animals*, Vol. 43, No 1, pp. 91–95, https://doi.org/10.1258/la.2008.007139.

Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals. http://data.europa.eu/eli/reg/2006/1907/oj.

Regulations (EC) 1/2005 on the protection of animals during transport and related operations. http://data.europa.eu/eli/reg/2005/i/ei.

Regulations (EU) 2016/429 on transmissible animal diseases. http://data.europa.eu/eli/reg/2016/429/oj.

Research Institute of Sweden, Housing, handling and training rats at RISE. https://3rc.org/rats-refinement.

Robinson, S. and Kerton, A. (2021), 'What does a culture of care look like? Lessons learnt from a workshop survey', LabAnimal, Vol. 50, No 10, pp. 269–271.

Royal Society for the Prevention of Cruelty to Animals (RSPCA) (2020), 'Resources for AWERB members – Promoting a culture of care', RSPCA website, www.rspca.org.uk/webContent/staticImages/Downloads/PromotingACultureOfCare.pdf.

Russell, W. M. S. and Burch, R. L. (1959; reprinted 1992), The Principles of Humane Experimental Technique, Universities Federation for Animal Welfare (UFAW), Wheathampstead.

Smith, D., Anderson, D., Degryse, A. D., Bol, C., Criado, A., et al. (2018), 'Classification and reporting of severity experienced by animals used in scientific procedures: FELASA/ECLAM/ESLAV Working Group report', Laboratory Animals, Vol. 52, pp. 5–57.

The 3Rs Collaborative (n.d.), 'Rodent health monitoring', The 3Rs Collaborative website, https://3rc.org/health-monitoring/.

The Jackson Laboratory, 'Breeding colony size planning worksheet', The Jackson Laboratory website, https://media.jax.org/m/468eb6d3f89b171a/original/breeding-colony-size-planning-work-sheet.pdf.

The Jackson Laboratory, 'Strain background and genetic drift', The Jackson Laboratory website, https://www.jax.org/jax-mice-and-services/customer-support/technical-support/strain-background-and-genetic-drift.

Tough, B. (2023), 'Rat playpens – The effect on welfare, technical time and budget', Animal Technology and Welfare, Vol. 22, No 2, pp. 156–157.

Turner, P. V., Brabb, T., Pekow, C. and Vasbinder, M. A. (2011), 'Administration of substances to laboratory animals: Routes of administration and factors to consider', Journal of the American Association of Laboratory Animal Science, Vol. 50, No 5, pp. 600–613.

Wang, J. Y., Doudna, J. A. (2023), 'Crispr technology: A decade of genome editing is only the beginning', *Science*, Vol. 379, No 6629, eadd8643, https://doi.org/10.1126/science.add8643.

Waynforth, B. (2007), 'Experimental procedures', in: Barnett, S. W. (ed.), Manual of Animal Technology, pp 351-364, Blackwell Publishing, Oxford.

WHO (World Health Organization) (2020), 'Zoonoses', WHO website, https://www.who.int/news-room/fact-sheets/detail/zoonoses.

Wolfensohn S and Lloyd M (1998), 'Handbook of Laboratory Animal Management and Welfare' 2nd ed., pp.75 Blackwell Science, Oxford

List of abbreviations

Abbreviations	Meanings
20-nt	20-nucleotide
ACH	Air changes per hour
AAV	Adeno-associated virus
ALURES	Animal Use Reporting EU System (EU statistics database on the use of animals for scientific purposes under Directive 2010/63/EU)
ATW	Animal Technology and Welfare
AWB	Animal welfare bodies
BMS	Building management system
BSE	Bovine spongiform encephalopathy
Cas9	Crispr-associated protein 9
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CJD	Creutzfeldt–Jakob disease
CO_2	Carbon dioxide
Crispr	Clustered regularly interspaced short palindromic repeats
dB	Decibels
DNA	Deoxyribonucleic acid
DV	Designated Veterinarian
ECHA	European Chemicals Agency
EFAT	European Federation of Animal Technologists
EHM	Environmental health monitoring
ETPLAS	Education and Training Platform for Laboratory Animal Science
EU	European Union
EU-OSHA	European Agency for Safety and Health at Work
FELASA	Federation of European Laboratory Animal Science Associations
G	Gauge (in this book refers to size of hypodermic needle)
GAA	Genetically Altered Animals
GLP	Good Laboratory Practice
HEPA filter	High-efficiency particulate air (filter)
HVAC	Heating, ventilation and air conditioning

Hz	Hertz (in this book refers to frequency of sound and light)
IAT	Institute of Animal Technology
IATA	International Air Transport Association
ISO	International Organization for Standardization
IVCs	Individually Ventilated Cages
LAAs	Laboratory animal allergens
LO	Learning Outcomes
mESCs	Mouse embryonic stem cells
MSDs	Musculoskeletal disorders
NC3Rs	National Centre for the Replacement, Refinement and Reduction of Animals in Research
OJ	Official Journal of the European Union
OSH	Occupational safety and health
PAM	Protospacer adjacent motif
PPE	Personal Protective Equipment
ppm	Parts per million
QACs	Quaternary ammonium compounds
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals (European Regulation (EC) No.1907/2006)
RH	Relative humidity
RISE	Research Institutes of Sweden
sgRNA	Single guide RNA
SOPs	Standard operating procedures
SpCas9	Streptococcus pyogenes crispr-associated protein 9
SPF	Specified (or specific) Pathogen Free
TBV	Total blood volume
Three Rs	Replacement, Reduction, Refinement
TSEs	Transmissible spongiform encephalopathies
UFAW	Universities Federation for Animal Welfare
UV	Ultraviolet
VHP	Vaporised hydrogen peroxide

List of figures

Figure 1.1. Percentages of animals used in the EU and Norway for the first time in 2023 14
Figure 1.2. Purposes of animal use in the EU and Norway in 2023 14
Figure 1.3. Severity classification 18
Figure 2.1. Terminology and prospective classification of severity 32
Figure 2.2. Severity assessment: a continuous process 34
Figure 3.1. Mouse Des Res 42
Figure 3.2. Rabbits in floor pens with tubes and boxes 43
Figure 3.3. Top of a marmoset sentry cage showing a nest box 43
Figure 3.4. Beagle with chew toy 44
Figure 3.5. Food puzzle 45
Figure 3.6. Dog trained to walk on a lead 45
Figure 4.1. Traumatic damage to tail 53
Figure 4.2. Overgrown teeth caused by malocclusion 53
Figure 4.3. Flexible film isolators. A = entry port; B = Filter for air extract 55
Figure 4.4. Block diagram of a laboratory animal facility 56
Figure 4.5. Disinfectant dunk tank straddling the barrier between the clean and dirty sides 57
Figure 4.6. Insect barrier 57
Figure 4.7. Rodent barrier 58
Figure 4.8. Air shower 59
Figure 4.9. Trolley covers 60
Figure 4.10. Healthy, alert rabbit in its cage 63
Figure 4.11. Healthy, alert rat in its cage 63
Figure 4.12. Normal teeth of a rabbit 65
Figure 4.13. Bottom incisor overgrown 65
Figure 4.14. Rabbit with respiratory disease. Note the discharge around the nose and the matted fur on the forepaws 66
Figure 4.15. Brown/yellow exudate in the inner ear indicating ear canker 67
Figure 4.16a. Normal zebrafish 69
Figure 4.16b. Fish with dropsy, showing enlarged abdomen and raised scales 69
Figure 5.1. Rabbit on a raised platform 75

Figure 5.2. Dog sitting on a raised shelf 75
Figure 5.3. Dogs being exercised in a facility corridor 76
Figure 5.4. Large non-human primate cage 79
Figure 5.5. Polycarbonate mouse cage 79
Figure 5.6. Individual Ventilated Cage 80
Figure 5.7. Racks of IVCs 80
Figure 5.8. Linked rat cage 81
Figure 5.9. Pig pen with scratching brush as environmental enrichment 82
Figure 5.10. Rabbit floor pen 82
Figure 5.11. Multi-rack system showing several connected racks for Zebrafish 83
Figure 5.12. Stand-alone system for Zebrafish 84
Figure 5.13. A Magnehelic pressure gauge, which shows that air pressure in the room is slightly higher than that in the corridor 87
Figure 5.14. Farm animal housing showing a solid wall base topped with weatherboarding. This arrangement allows an adequate airflow above the animals while protecting them from draughts ... 88
Figure 5.15. Agama lizard basking in the morning sun 88
Figure 5.16. Ringtail in rats 90
Figure 5.17. Dog unit fitted with glass bricks to let in daylight while maintaining security 92
Figure 5.18. Light meter reading 419 lux 92
Figure 5.19. Pink eyes of an albino rat, indicating lack of retinal pigment 93
Figure 5.20. Sound meter reading 58.2 dBA 95
Figure 5.21. BMS screen. Right: a section of the BMS screen showing environmental levels for animal rooms. Left: a small section of a BMS screen showing warning of some environmental readings outside set levels 97
Figure 5.22. Thermo-hygrograph 98
Figure 5.23. Small Electronic Thermo-hygrograph 98
Figure 6.1. Cage-cleaning station 108
Figure 6.2. Airflow through a cage-cleaning station 109
Figure 6.3. Robot working in a cage wash area 110
Figure 6.4. Cover on a trolley carrying dirty cages 110
Figure 6.5. Tunnel washer 111
Figure 6.6. Cabinet washer 112
Figure 6.7. Rack washer 112

Figure 6.8. Cages arranged on a rack ready to go into a rack washer 113
Figure 6.9. Bottle-cleaning and -filling station 114
Figure 6.10. Wood chips 116
Figure 6.11. Wood shavings 116
Figure 6.12. Paper sheet used as a non-contact bedding 117
Figure 6.13. Cellulose-based bedding material 117
Figure 6.14. Paper shavings 118
Figure 6.15. Corncob 118
Figure 6.16. Young rabbit in a nest made with hay and lined with the doe's fur 119
Figure 6.17. Cotton fibre nesting material, which rodents are able to shred to make nests 120
Figure 6.18. Bitch with pups on Vetbed™ 120
Figure 6.19. Loaded VHP decontamination unit 122
Figure 6.20. Fogger 122
Figure 6.21. Dry heat steriliser 123
Figure 6.22. Large autoclave 125
Figure 6.23. Small benchtop autoclave 126
Figure 6.24. Autoclave indicator tape. Top: appearance before autoclaving. Bottom: appearance after autoclaving 127
Figure 6.25. Indicator strip. The yellow dot changes to blue after the temperature has been reached for the required amount of time 127
Figure 7.1. A cast of the four-chambered stomach of a cow, viewed from the right side. In life, the rumen fills most of the left side of the abdomen 136
Figure 7.2. Compression pellets (left) and expanded pellets (right) 140
Figure 7.3. Mouse cage showing hopper 144
Figure 7.4. Rabbit hopper and water bottle 144
Figure 7.5. Food bowl on the floor 145
Figure 7.6. Mould 147
Figure 7.7. Flour mite 148
Figure 7.8. Flour moth 148
Figure 7.9. Grain weevil 149
Figure 7.10. Damaged grain 149
Figure 7.11. Sawtoothed grain beetle 150

Figure 7.12. Water bag in a mouse cage 151

Figure 7.13. Apparatus for filling and sealing water bags 152

Figure 7.14. Rat drinking from an automatic water nipple 152

Figure 7.15. Pig farrowing unit showing drinking nipples on the left of the opening. Note the two heights, one for the sow and one for the piglets 153

Figure 8.1. Vaginal plug in a mouse 160

Figure 8.2. Inbred mouse CBA/ca (dark); outbred mouse CD-1 of the same age 161

Figure 8.3. A one- or two-day-old mouse pup showing closed eyes and ears and baldness typical of altricial young 161

Figure 8.4. Rat vaginal plugs 162

Figure 8.5. Post-ovulatory discharge 163

Figure 8.6. Young guinea pigs, fully furred and active, typical of a precocial species 164

Figure 8.7. Young rabbits in hay nest lined with fur from the mother 165

Figure 8.8. Litter of ferrets 166

Figure 8.9. Beagles tied during mating 167

Figure 8.10. Beagle pup in a Vetbed nest 167

Figure 8.11. Mesh insert in a production tank (the minimum water volume must be at least 1 litre) 168

Figure 8.12. Breeding box of nude mice. A = heterozygous pups (hairy); B = homozygous pups; C = heterozygous female (hairy); D = homozygous male (nude) 173

Figure 8.13. Rat nest 177

Figure 8.14. A one-day-old rat. Eyes closed, pinnae close to the head, no hair on body 178

Figure 8.15. An 11-day-old rat. Eyes closed, pinnae separating from head, body covered with short hair 178

Figure 8.16. A 19-day-old rat. Fully furred, ears separate from head 179

Figure 8.17. A 9- to 10-day-old rabbit 180

Figure 8.18. A 25-day-old kitten. Eyes open, but ears still not fully separated 180

Figure 8.19. A 7-day-old beagle puppy 181

Figure 9.1. Schematic of Crispr/SpCas9 creating a double-stranded DNA break in zygotes. (a) Complexing of an sgRNA with SpCas9. (b) Electroporation of the sgRNA/SpCas9 complex into mouse zygotes. (c) Complementary base pairing of the sgRNA with the genomic target DNA. (d) SpCas9 cuts the target DNA 3–5 bp 5′ of the PAM site. (3-5 bp is the DNA target and PAM (protospacer-adjacent motif), is used to mark/identify the proper target site) 188

Figure 10.1. Rabbits with variation in coat colour housed together 192

Figure 10.2. Identification stains on guinea pigs 194

Figure 10.3. Chicken leg rings 194
Figure 10.4. Ear punch 195
Figure 10.5. Transponder reader 196
Figure 10.6. Transponders and applicator 196
Figure 10.7. Ketcham tags with pliers 197
Figure 10.8. Assorted Rototags and one Ketchum tag 197
Figure 10.9. Young rats fitted with ear studs 198
Figure 10.10. Ear tattoo on a rabbit 199
Figure 10.11. Tattoo pen 199
Figure 11.1. Transport box for small laboratory animals showing viewing window 203
Figure 11.2. Butterfly needle 205
Figure 11.3. Inserting a needle into a rabbit's ear vein 208
Figure 11.4. Vacuum system for blood collection, showing exterior needle, barrel and vacuum tube 210
Figure 11.5. A metabolism cage 212
Figure 11.6. Diagram of rodent metabolism cage 213
Figure 12.1. Captive bolt gun 221
Figure 12.2. A euthanasia cabinet for use with carbon dioxide 222
Figure 12.3. A carbon dioxide euthanasia system designed to hold a specific cage 223
Figure 13.1. Picking up a mouse by the tail 228
Figure 13.2. Moving mice by cupping in the hand 228
Figure 13.3. A mouse being guided into a plastic tube 229
Figure 13.4. Restraining a mouse 229
Figure 13.5. A one-day-old female mouse 230
Figure 13.6. A one-day-old male mouse 230
Figure 13.7. A 10-day-old female mouse 230
Figure 13.8. A 10-day-old male mouse 230
Figure 13.9. Weaner mice, female (left) and male (right) 231
Figure 13.10. Adult mice, male (left) and female (right) 231
Figure 13.11. Picking up a rat 232
Figure 13.12. Restraining a rat 232
Figure 13.13. Female rat, one or days old 233

Figure 13.14. Male rat, one or two days old 233
Figure 13.15. Female rat, 10 days old 234
Figure 13.16. Male rat, 10 days old 234
Figure 13.17. Female rat, 19 days old 234
Figure 13.18. Male rat, 19 days old 234
Figure 13.19. Adult female rat 234
Figure 13.20. Adult male rat 234
Figure 13.21. Cupping a hamster 235
Figure 13.22. Picking up a hamster 235
Figure 13.23. Male hamster 236
Figure 13.24. Female hamster 236
Figure 13.25. Holding a guinea pig 236
Figure 13.26. Adult female guinea pig 237
Figure 13.27. Adult male guinea pig 237
Figure 13.28. Immature female guinea pig 237
Figure 13.29. Immature male guinea pig 237
Figure 13.30. Rabbit about to be put into a transport box 238
Figure 13.31. Removing a rabbit from the cage 238
Figure 13.32. Rabbit in a carrying position 239
Figure 13.33. Holding a rabbit for sexing 240
Figure 13.34. Immature female rabbit 241
Figure 13.35. Immature male rabbit 241
Figure 13.36. Adult female rabbit 241
Figure 13.37. Adult male rabbit 241
Figure 13.38. Holding a ferret 242
Figure 13.39. Female ferret 242
Figure 13.40. Male ferret 242
Figure 13.41. Female kitten 243
Figure 13.42. Male kitten 243
Figure 13.43. Adult female kitten 243
Figure 13.44. Adult male kitten 243

Figure 13.45. Lifting a male dog 244
Figure 13.46. Male puppy 244
Figure 13.47. Female puppy 244
Figure 13.48. Adult female (bitch) 245
Figure 13.49. Zebrafish restrained on a sponge 246
Figure 13.50. Adult zebrafish male (top), female (bottom) 246
Figure 14.1. Sharps bin 252
Figure 14.2. The colour codes of the major types of hazard warning signs 254
Figure 14.3. Hazard pictograms 256
Figure 14.4. Winchester carriers 257
Figure 14.5. A carbon dioxide (CO^2) fire extinguisher showing the black panel and instructions for use 258

List of tables

Table 4.1. Examples of non-infectious diseases 52

Table 4.2. Categories of animals kept in bio-exclusion facilities 54

Table 4.3. Biological agent hazard groups 61

Table 4.4. Signs that could indicate ill health 64

Table 4.5. Ignored or excessive intake 64

Table 4.6. Health checklist for a mouse 68

Table 4.7. Some signs of ill health in zebrafish 69

Table 4.8. Examples of stress-inducing factors 70

Table 5.1. Properties of common caging materials 77

Table 5.2. Suitable temperature ranges for adult uncompromised animals 89

Table 5.3. Examples of the effects of temperature and RH variation on experimental results 91

Table 5.4. Suggested RH (%) for laboratory animals 91

Table 6.1. Stages in the cage-washing process 113

Table 6.2. Steam sterilisation: relationship between temperature, pressure and time 124

Table 6.3. Stages in the autoclave cycle 125

Table 7.1. Essential and non-essential amino acids used to construct proteins 135

Table 7.2. The parts of a cow's four-chambered stomach (from **Figure 7.1**) 136

Table 7.3. Fat-soluble vitamins 137

Table 7.4. Water-soluble vitamins 137

Table 7.5. Food and water consumption per adult male per day 140

Table 7.6. Methods of presenting water 153

Table 8.1. Glossary of breeding terms 156

Table 8.2. Mating arrangements 159

Table 8.3. Summary of breeding data of common laboratory species 176

Table 8.4. Stages of physical development in outbred mice and rats 178

Table 8.5. Approximate ages and weights of outbred animals at different stages of development 181

Table 9.1. Glossary of biological terms used in Chapter 9 185

Table 11.1. Maximum injection volumes (ml/kg of body weight) 208

Table 11.2. Total blood volumes 209

Table 12.1. Methods of euthanasia to be used on laboratory animals 218

Table 14.1. Symptoms of laboratory animal allergy 250

Table 14.2. Examples of Fire extinguishers and their uses 259

Table 15.1. Mapping Manual Chapters to Modules and Learning Outcomes 264

Table 15.2. Mapping Modules and Learning Outcomes to Manual Chapters 265

Getting in touch with the EU

In person

All over the European Union there are hundreds of Europe Direct centres. You can find the address of the centre nearest you online (european-union.europa.eu/contact-eu/meet-us_en).

On the phone or in writing

Europe Direct is a service that answers your questions about the European Union. You can contact this service:

— by freephone: 00 800 6 7 8 9 10 11 (certain operators may charge for these calls),
— at the following standard number: +32 22999696,
— via the following form: european-union.europa.eu/contact-eu/write-us_en.

Finding information about the EU

Online

Information about the European Union in all the official languages of the EU is available on the Europa website (europa.eu).

EU publications

You can view or order EU publications at op.europa.eu/en/publications. Multiple copies of free publications can be obtained by contacting Europe Direct or your local documentation centre (european-union.europa.eu/contact-eu/write-us_en).

EU law and related documents

For access to legal information from the EU, including all EU law since 1951 in all the official language versions, go to EUR-Lex (eur-lex.europa.eu).

Open data from the EU

The portal data.europa.eu provides access to open datasets from the EU institutions, bodies and agencies. These can be downloaded and reused for free, for both commercial and non-commercial purposes. The portal also provides access to a wealth of datasets from European countries.

Science for policy

The Joint Research Centre (JRC) provides independent, evidence-based knowledge and science, supporting EU policies to positively impact society

Scan the QR code to visit:

Joint Research Centre
https://joint-research-centre.ec.europa.eu

Publications Office
of the European Union
Law | Data | Publications