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SOUTH AFRICAN NATIONAL STANDARD

The care and use of animals for scientific purposes

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Edition 1

Table of changes

Change No.	Date	Scope

Acknowledgement

This South African standard is based on the Australian Standard of Practice for the care and use of animals for scientific purposes, September 1997, drawn up by the National Health and Medical Research Council of Australia (copyright Commonwealth of Australia, reproduced by permission), and on the European Convention for the protection of vertebrate animals used for scientific study and for other scientific purposes.

Foreword

This South African standard was approved by National Committee SABS SC 1040D, *Steering committee for nature conservation — The care and use of animals for scientific purposes*, in accordance with procedures of the SABS Standards Division, in compliance with annex 3 of the WTO/TBT agreement.

This document was published in December 2008.

Annexes A to P are for information only.

Introduction

The purpose of this standard is to ensure the ethical and humane care of animals used for scientific purposes, as well as for teaching activities. Its aims are to:

- a) emphasise the responsibilities of researchers, teachers and institutions using animals;
- b) ensure that the welfare of animals is always considered;
- c) ensure that the use of animals is justified by the establishment of Animal Ethics Committees (AECs) so as to ensure adherence to the principles of Replacement, Reduction and Refinement (the 3 Rs);
- d) prevent or minimize pain or distress, where possible, for each animal used in scientific studies and teaching activities;
- e) ensure minimum uniform national standards regarding animal care and use;
- f) minimize the number of animals used in scientific studies and teaching activities in such a way that this does not jeopardise the validity of the studies or activities; and
- g) promote the development and use of techniques which adhere to the principles of Replace, Reduce and Refine animal use in scientific studies and teaching activities (see (c)).

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The care and use of animals for scientific purposes

1 Scope

This standard encompasses all aspects of the care and use of, or interaction with, animals for scientific purposes in medicine, biology, agriculture, veterinary and other animal sciences, as well as industry and teaching studies in South Africa. It includes animal use in research, teaching, field trials, product testing, diagnosis, the production of biological substances and environmental studies.

It provides general principles for the care and use of animals, specifies the responsibilities of researchers and institutions, and details the terms of reference, membership and operation of institutional Animal Ethics Committees (AECs). It also provides guidelines for the humane conduct of scientific studies and teaching activities, and for the acquisition of animals and their care, including their environmental needs. Where applicable, this standard is intended to be used as a supporting document to be read in conjunction with the Animals Protection Act, 1962 (Act No. 71 of 1962), the Animal Diseases Act, 1984 (Act No. 35 of 1984), the Veterinary and Paraveterinary Professions Act, 1982 (Act No. 19 of 1982) and the subsequent Notice 1445 of 1997, published in the Government Gazette 18313 of 3 October 1997: *Rules relating to the practising of the Paraveterinary professions of Laboratory Animal Technologists*, the Animal Health Act, 2002 (Act No. 7 of 2002), the Medicines and Related Substances Control Act, 1965 (Act No. 101 of 1965) and the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act No. 36 of 1947) and any other relevant provincial and national legislation.

This standard covers all live non-human vertebrates and higher invertebrates such as the advanced members from the *Cephalopoda* and *Decapoda*. It also covers eggs, fetuses and embryos and their treatment in a humane manner where development of an integrated nervous system is evident.

NOTE Researchers should forward proposals to use lower order invertebrates to AECs.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies. Information on currently valid national and international standards can be obtained from the SABS Standards Division.

2.1 Standards

SANS 10331 (SABS 0331), *Translocation of certain species of wild herbivore*.

2.2 Other publications

Canadian Council on Animal Care (CCAC). 1993. *Guide Volume 1*, 2nd Edition.

Available at:

http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/guides/english/v1_93/chap/chiii.htm.

International Air Transport Association (IATA). 2008/2009. *Live Animal Regulations (LAR)*, English manual.

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Working Party EU Commission Directive. 1997. *Recommendations for Euthanasia of Study Animals*. Working Party Report, EU Commission Directive 86/609/EEC of 24 November 1986.

Livestock Welfare Co-ordinating Committee (LWCC), University of Pretoria Faculty of Veterinary Science Onderstepoort. 2003. *Optimising animal welfare when carrying out standard farm procedures and operations on livestock*.

Available at: <http://www.samic.co.za/SAMIC/LWCC.htm>.

Wildlife Decision Support Services. *The Capture and Care Manual*, 1983. Edited by McKenzie, A. The Wildlife Decision Support Services and South African Veterinary Foundation, Pretoria.

3 Definitions

3.1

animal

live, sentient non-human vertebrate, including eggs, foetuses and embryos, that is; fish, amphibians, reptiles, birds and mammals, and encompassing domestic animals, purpose-bred animals, farm animals, wildlife (see 3.28) and higher invertebrates such as the advanced members from the *Cephalopoda* and *Decapoda*

3.2

Animal Ethics Committee

AEC

committee constituted in accordance with the terms of reference and membership laid down in this standard

3.3

animal studies

procedure that uses animals for one or more of the following reasons:

- a) for the advancement of knowledge;
- b) to test a hypothesis;
- c) to supply a product other than cosmetic products;
- d) to produce a biological substance;
- e) to provide tissues, organs or serum;
- f) to act as a host;
- g) to impart or demonstrate existing knowledge;
- h) to teach or learn surgical techniques; and
- i) to fulfil statutory requirements for the testing or collecting of data on a substance or product.

3.4

approved study

study which has been formally approved by a properly constituted AEC on the basis of a written proposal

3.5

distress

acute or chronic response of an animal caused by stimuli that produce biological stress which manifests as observable, abnormal physiological or behavioural responses

3.6

environmental enrichment

measures taken to enable species-specific behaviour, to alleviate boredom and to eliminate abnormal or harmful behaviour

3.7

euthanasia

act or practice of ending the life of an animal as painlessly and humanely as possible

3.8

farm animals

animals which are used in commercial agriculture, including cattle, sheep, pigs, poultry, goats, horses, fish and wildlife that is intensively farmed

3.9

feral

existing in a wild state, after having been domesticated

3.10

humane endpoint

deliberate measure of using the death of an animal(s) for evaluating biological or chemical processes, responses or effects, or based on humane reasons to alleviate pain, suffering and distress

3.11

institution

entity that uses animals for scientific purposes and teaching studies

3.12

Institutional Biological Safety Committee

IBSC

committee charged with reviewing proposed use of biohazardous agents, human material, and recombinant deoxyribonucleic acid (DNA) molecules to assess compliance with applicable regulatory guidelines

3.13

laboratory animals

animals or a group of animals with a known genetic or microbiological composition (or both) (see annex A)

3.14

laboratory animal science

multi-disciplinary branch of science that contributes to the humane use of animals in biomedical research and the collection of informative, unbiased and reproducible data

NOTE Laboratory animal science encompasses the study of the biology of laboratory animals, their husbandry and environmental requirements, genetic and microbiological standardization procedures, prevention and treatment of diseases, optimisation of study techniques and improvement of anaesthesia, analgesia and euthanasia.

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3.15

modifying animal behaviour

procedures used to alter an animal's behaviour or to induce it to perform specific tasks

3.16

pain

awareness of acute or chronic discomfort that occurs in varying degrees of severity as a result of study procedures, injury, disease or confinement

3.17

person-in-charge

person who, by appointment or delegation, is in charge of an animal facility and has total control and responsibility for the care and wellbeing of animals in the facility

3.18

proposal

written outline of a scientific study or teaching activity submitted to an AEC for consideration

3.19

rehabilitation

preparation of an animal for release into its natural environment

3.20

researcher

person recognized by an AEC as competent to conduct an approved scientific study that involves animals

3.21

scientific study

study or a series of related studies that form a discrete piece of work, performed to acquire and develop knowledge or techniques in any scientific discipline, including studies for the purposes of research, diagnosis, product testing, field trials, environmental studies, and the production of biological substances

3.22

sentient

having the power of sense perception or sensation

3.23

stereotypic behaviour

repetitive sequence of movements without an apparent goal which are derived from normal maintenance behaviour but appear either out of context, exaggerated or unusually sustained

3.24

teacher

person recognized by an AEC as competent to conduct an approved teaching activity that involves animals

3.25

teaching activity

activity that involves animals and is performed to acquire, develop or demonstrate knowledge or techniques in any scientific discipline, including studies for the purposes of teaching and training in primary, secondary and tertiary institutions

3.26

voucher specimen

specimen which serves as a basis of study and is retained as a reference

3.27

wellbeing

welfare

tendency towards a relatively stable equilibrium between interdependent elements, especially as maintained by physiological and psychological processes

3.28

wildlife

indigenous or non-indigenous self-sustaining species of animals, whether domesticated or not

4 General principles for the care and use of animals for scientific studies and teaching activities

4.1 Purpose

The purpose (see introduction) is to ensure that in scientific studies and teaching activities there is:

- a) Replacement of the use of animals with other methods;
- b) Reduction in the number of individual animals used; and
- c) Refinement of techniques used to reduce the impact on individual animals.

4.2 Justification and responsibilities

4.2.1 Scientific studies and teaching activities that use animals may be performed only when they are essential to:

- a) obtaining, establishing and disseminating significant information and knowledge relevant to the understanding of humans or animals;
- b) maintaining and improving human or animal health and welfare; and
- c) improving animal management or production.

4.2.2 Researchers and teachers shall submit written proposals for all animal studies to an AEC which shall take into account the expected value of the knowledge to be gained, the justification for the study, and all ethical and animal welfare aspects.

4.2.3 Scientific studies and teaching activities that use animals may only be performed when appropriate non-animal alternative methods are not available. Proof shall be provided on request of the AEC that non-animal alternatives have been sought.

4.2.4 Scientific studies or teaching activities that use animals may be performed only after a decision has been made by the AEC that they are justified, through weighing the scientific or educational value of the scientific study or teaching activity against the potential effects on the welfare of the animals.

4.2.5 Scientific studies and teaching activities shall not commence until written approval has been obtained from the AEC. Failure to obtain such approval might result in scientific studies or teaching activities not being recognized.

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4.2.6 People who use animals for scientific purposes have an obligation to treat animals with respect and to ensure their welfare as an essential factor when planning and conducting studies. Researchers and teachers have direct and ultimate personal responsibility for all matters relating to the welfare of the animals they use.

4.2.7 The acquisition, care and use of animals for all scientific purposes in South Africa shall be in accordance with this standard and with the Animals Protection Act, 1962 (Act No. 71 of 1962).

4.2.8 Institutions that use animals for scientific purposes shall establish AECs to ensure that all animal use conforms to the precepts of this standard (see 5.1).

4.3 Replacement

Techniques which replace or complement the use of animals in scientific studies and teaching activities shall be sought and used wherever possible.

4.4 Reduction

4.4.1 Scientific studies and teaching activities shall be scientifically and statistically valid, shall use only the minimum number of animals necessary, and shall not be repeated unnecessarily.

4.4.2 The principle of reducing the number of animals used in scientific studies and teaching activities shall not be implemented at the expense of the greater suffering of individual animals.

4.4.3 Production of animals bred for scientific purposes or for breeding programmes shall be rationalized to prevent overproduction of animals so as to reduce the number of healthy animals put to death by recognized euthanasia methods.

4.5 Refinement

4.5.1 Animals chosen for scientific studies and teaching activities shall be suitable for the purposes of the investigation taking into account their biological characteristics, including behaviour, genetic constitution and nutritional, microbiological and general health status. The welfare of the animals shall be a primary consideration in the provision of care and shall be based on the behavioural and biological needs of the species.

4.5.2 Wildlife shall only be taken from their natural habitat if animals bred in captivity are unsuitable or unavailable for the specific scientific purpose, and then only in accordance with the Provincial Nature Conservation Ordinances and Biodiversity Act, 2004 (Act No.10 of 2004).

4.5.3 Researchers and teachers shall use the best available scientific techniques and shall be certified as competent in the procedures they perform by the AEC (see 5.2.4.3 (b) and (c)).

4.5.4 Scientific studies and teaching activities shall be so designed as to avoid pain or distress to animals. If this is not possible, pain or distress shall be minimized.

4.5.5 Since pain and distress cannot be evaluated easily in animals, researchers and teachers shall assume that animals experience pain and distress in a manner similar to humans. Decisions regarding the animal's welfare shall be based on this assumption unless there is evidence to the contrary.

4.5.6 An animal that develops signs of pain or distress of a kind and degree not predicted in the proposal shall have the pain or distress alleviated promptly. If severe pain cannot be alleviated promptly, the animal shall be put to death by recognized euthanasia methods forthwith. Alleviation of such pain or distress shall take precedence over finishing a study.

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NOTE The scientific report, entitled *Aspects of the biology and welfare of animals used for experimental and other scientific purposes* of the European Food Safety Authority (Annex to the EFSA Journal (2005) 292, 1 - 136), is recommended as a guideline.

4.5.7 Scientific studies and teaching activities that might cause pain or distress of a kind and degree for which anaesthesia would normally be used in medical or veterinary practice shall be carried out by an authorized, competent and experienced person using anaesthesia appropriate to the species and the procedure.

NOTE The use of analgesic, sedative and tranquillising agents should at least parallel usage in medical or veterinary practice.

4.5.8 Pain management procedures appropriate to the species and the circumstances shall be provided.

4.5.9 When it is not possible to use anaesthetics or analgesics, such as in certain toxicological or animal production studies or in animal models of disease or challenge testing of vaccines, care shall be taken that the endpoint of the study shall be as early as possible to avoid or to minimize pain or distress to the animals.

4.5.10 Neuromuscular blocking agents shall not be used without appropriate general anaesthesia except in animals where sensory awareness has been eliminated. If such agents are used, continuous or frequent intermittent monitoring of paralysed animals shall be essential to ensure that the depth of anaesthesia is adequate to prevent pain or distress.

4.5.11 Researchers shall avoid using death as a study endpoint whenever possible.

NOTE The *Recommended techniques of choosing an appropriate endpoint* of the Canadian Council on Animal Care Guideline Policies and the annex on pain management and humane endpoints in this standard may be consulted.

4.5.12 Scientific studies and teaching activities that involve the use of animals shall be as brief as possible.

4.5.13 Animals shall be transported, housed, fed, watered, handled, bred and used under conditions that are appropriate to the care and wellbeing of the species as well as of individual animals.

4.5.14 An assessment of the potential sources of stress and management plans to eliminate or minimize distress shall form part of every proposal submitted to the AEC. Assessment scoring sheets shall also be submitted.

5 Responsibilities of institutions and their AECs

5.1 Responsibilities of institutions

Institutions that use animals for scientific studies and teaching activities shall:

- a) establish one or more AECs comprising members who are directly responsible to the governing body of the institution or its delegate. Where animal use is small, the institution may access an external AEC;
- b) ensure, through the AEC, that all scientific studies and teaching activities that involve the use of animals comply with relevant legislation, including, in particular, compliance with Section 23 (1) (C) of the Veterinary and Paraveterinary Professions Act, 1982 (Act No. 19 of

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1982) which states that "only people with the correct Section 23 (1) (C) authorization under the said Act may carry out procedures specific to the veterinary profession";

- c) provide each AEC with facilities, powers and resources to fulfil its terms of reference and operation as set out in 5.2;

NOTE Provision of resources should include areas such as orientation of new AEC members, purchase of educational material, access to training courses for AEC members and access to administrative assistance.

- d) refer to the appropriate AECs for comment on all matters that might affect animal welfare in the institution;
- e) have an internal review annually and an external review every three years of the operation of each AEC;

NOTE This review should include assessment of the annual report from the AEC and a meeting with the AEC Chairperson.

- f) respond effectively to recommendations from each AEC to ensure that the acquisition of animals, and the facilities for the housing, care (see annexes B to N), use and disposal of animals are appropriate to the maintenance of the health and wellbeing of the animals;

NOTE The AEC should report through the Chairperson to the Chief Executive Officer (CEO) of the institution (or the delegated representative of the CEO) and, when fulfilling its responsibilities, should receive the full support of the CEO.

- g) respond promptly and effectively to recommendations from each AEC to ensure that all use of animals for scientific purposes within the institution remains in accordance with this standard;
- h) following an investigation by and upon the advice of the AEC, discipline staff who contravene the requirements of this standard;
- i) provide all relevant staff with details of the institution's policy on the care and use of animals, confidentiality, freedom of information, legislation, legal requirements and commercial considerations;
- j) provide staff members with information on potential disease hazards from their work with animals and engage in pro-active and preventative measures (for example, periodical examinations of persons working with animals), provide vaccinations where and when appropriate (for example, rabies, tetanus, hepatitis A and hepatitis B), and provide staff members and researchers with personal protective devices (PPDs) where and when appropriate;
- k) establish mechanisms to respond to enquiries or complaints concerning the use of animals within the institution and ensure that staff members are able to voice concerns without jeopardising their employment;
- l) establish grievance procedures for AEC members and researchers who are dissatisfied with the AEC's procedures or decisions;
- m) ensure that the AEC develops guidelines for animal care and use within the institution and that these are implemented, including those which ensure that emergencies are detected promptly and are dealt with effectively;
- n) ensure that there is an adequate number of staff members competent and trained to care for the animals;

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- o) ensure that relevant veterinary services are available and that there is access to diagnostic services;
- p) ensure that sufficient funds and resources are made available to meet the financial needs of laboratory animal facilities to ensure the proper care of animals and the management of the institutional laboratory animal facility; and
- q) have and provide the following:
 - 1) an organizational chart;
 - 2) defined lines of responsibility;
 - 3) key institutional staff;
 - 4) accreditation status;
 - 5) review or audit history;
 - 6) list of satellite facilities or contract facilities;
 - 7) defined processes for appointment of AEC members and the AEC Chairperson;
 - 8) clearly defined disciplinary procedures for any non-compliance with this standard;
 - 9) clearly defined Occupational Health and Safety programme(s) that are in-line with the Occupational Health and Safety Act, 1993, (Act No. 85 of 1993); and
 - 10) an Institutional Biological Safety Committee (IBSC) (see 3.12) and officers.

5.2 Responsibilities and operation of AECs

5.2.1 Compliance with this standard

AECs shall ensure that all animal care and use within the institution is conducted in compliance with this standard and incorporates the principles of Replacement, Reduction and Refinement, and provides for the welfare of the animals and justification for the use of animals in studies.

5.2.2 Terms of reference

AECs shall have terms of reference which include provisions to:

- a) approve only those studies for which animals are essential and which conform to the requirements of this standard, taking into consideration ethical and welfare aspects as well as scientific or educational value;
- b) monitor, inspect and assess the acquisition, transportation, production, housing, care, use and disposal of animals (see 5.2.7);
- c) communicate to the institution and require implementation by the institution any measures needed to ensure that the requirements of this standard are maintained;
- d) examine and approve, subject to modification, or reject written proposals relevant to the use of animals in scientific studies and teaching activities;
- e) formally withdraw approval for a study, or authorize the treatment or humane killing of an animal;

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- f) examine and comment on all institutional plans and policies which might affect animal welfare;
- g) maintain a register of approved studies;
- h) perform all other duties required by this standard;
- i) prepare written procedures, which are agreed to by the institution, to deal with non-compliance, research misconduct, and all other grievances related to the AEC process. The written procedures shall clearly define the reporting mechanisms and responsibilities of all parties to ensure fair and effective processes; and
- j) initiate investigation into any suspected or alleged non-compliance with this standard, institutional policies or the Animals Protection Act, 1962 (Act No. 71 of 1962).

NOTE It is not the function of the AEC to promote either side of the vivisection-antivivisection argument or to lobby for legislation.

5.2.3 Membership

5.2.3.1 An AEC shall have a membership that will allow it to fulfil its terms of reference. It shall comprise of at least four persons, one of which shall be independent of the institution, appointed in each of the following categories (see 5.2.3.5):

Category A. A person(s) with qualifications in veterinary science, with experience relevant to the studies of the institution or, in special circumstances, a person(s) with qualifications and experience to provide comparable expertise.

NOTE The intention is that every AEC has a veterinarian as the Category A member, however, since this might not always be possible for AECs in remote or very specialized research institutions (for example, in wildlife research), a well-trained wildlife officer might be an appropriate Category A member in such a case. Similarly, a person with qualifications in wildlife biology and experience with the species being studied might be the best Category A member in some very specialized research institutions.

Unless special circumstances exist, the AEC Category A position shall be filled by a veterinarian who shall have training and experience in the husbandry of the species being studied by the institution. Where veterinarians do not have this experience, they shall familiarize themselves with the biology and clinical characteristics of the species of animals being studied.

Category B. A person(s) with substantial recent experience in the use of animals in scientific studies or teaching activities.

NOTE The Category B member should have appropriate research or teaching experience. This will usually entail the possession of a higher degree(s).

Category C. A person(s) with demonstrable commitment to and established experience in furthering the welfare of animals, who is not employed by or otherwise associated with the institution, and who is not involved in the care and use of animals for scientific purposes. The person should, where possible, be selected on the basis of active membership of and nomination by an animal welfare organization.

NOTE When choosing the Category C member, a minimum of two nominees, nominated by separate animal welfare organizations, should be considered. It is preferable that the Category C person be a nominee, but not a representative, of an animal welfare organization since this will ensure the genuineness of the member's animal welfare credentials and that the member receives some measure of support from their nominating organization. It is for these reasons that this standard suggests that the Category C member be chosen on the basis of active membership of an animal welfare organization and has a track record in paid or voluntary work for the welfare of animals. The purpose of including an animal welfare person in each AEC is twofold:

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- a) This member should bring an animal welfare perspective to AEC deliberations. While all members of AECs shall consider the welfare of the animals, the Category C member brings to the committee a special awareness of current community animal welfare concerns and these will be his or her primary focus during AEC deliberations.
- b) Inclusion of animal welfare organizations countrywide will ensure that the animal welfare movement becomes knowledgeable about the work being performed in research and teaching institutions and that scientists and teachers become more aware of genuine concerns pertaining to animal welfare. Veterinarians are only appropriate as Category C members when they have specific animal welfare experience.

Category D. An independent person(s) who does not currently and has not previously conducted scientific studies or teaching activities using animals, and who is not an employee of the institution, except under defined circumstances (for example, tenured academic staff from non-scientific departments). If such an employee is appointed, the individual shall be in a senior position and shall not be supervised by other members of the committee or by anyone involved in animal research at the institution. The institution shall provide clear reasons for the necessity to appoint an employee in this category.

NOTE The Category D member should not fit any of the other categories (i.e. they should not be a veterinarian, should not have present or past research or teaching experience using animals, and should not qualify as an animal welfare member). They should be members of the wider community who can contribute different and independent perspectives to the AEC deliberations. It is envisaged that the Category D member will have no other association with the institution apart from his or her membership of the AEC. The wording says "except under defined circumstances" to cater for the special situation that exists at universities where tenured academic staff from non-scientific departments can be seen as being truly independent of the departments where medical or scientific research is undertaken. Other than this given specific situation, appointments to Category D should not be made internally, therefore, secretaries or administrative staff are deemed not suitable. Persons closely associated professionally with the institutions are also deemed not suitable. The Category D member should be viewed by the wider South African community as bringing a completely independent view to the committee and might include people such as distinguished public figures, business people, teachers, retirees, accountants, and lawyers.

5.2.3.2 A staff member responsible for the procurement, production, daily care and maintenance of the animals shall attend the AEC meetings.

NOTE It is essential to have someone who can provide the AEC with specific information on animals in the institution. It is not possible to make this a mandatory Category since in some circumstances the need for animal care might be minimal.

5.2.3.3 The AEC may include additional members to ensure that it can function effectively and to ensure that the social and psychological needs of animals are catered for via appropriate housing facilities and environmental enrichment. This shall be compulsory where high order mammals and primates are held at a facility (see annexes B to N).

NOTE Additional to the four required categories, institutions may appoint people with skills and background that can add value to the AEC (for example, bioethicists, biometricians, statisticians, behavioural biologists and ethologists). While there is no limit to the membership of AECs, it is important that there is a reasonable balance between the various categories and a reasonable balance between members from within the institution and members from outside. This standard requires that Category C and Category D represent no less than one third of the committee (see 5.2.3.5). In addition, AECs may co-opt people from time to time to provide expertise on specific issues, or to seek written advice.

5.2.3.4 Unless directed by internationally accepted study guidelines, the Chairperson shall hold a senior position in the institution.

NOTE It is considered advantageous for the Chairperson of an institutional AEC to have a senior position in the organization to ensure that recommendations made by the AEC are implemented quickly and effectively. A Chairperson based outside an institution might also be more difficult to contact in emergencies. However, in recognition that exceptions might be entirely satisfactory, for example, a senior person with managerial skills

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and access to and support from the institution's management might be appropriate, such as in the case of a small institution where the senior positions are filled by people who also conduct the bulk of the research.

5.2.3.5 If the committee has more than four members, Categories C plus D should represent no less than one third of the members.

5.2.3.6 Before appointment, all members of the AEC shall acknowledge in writing their acceptance of the terms of reference of the committee and any requirements for confidentiality required by the institution. The committee shall reach agreement on how advice may be sought without breaching confidentiality.

5.2.3.7 The AEC Chairperson shall have the power to immediately suspend or terminate any study, manipulation, or series of studies, where the Chairperson considers that any relevant legislation is being breached. The Chairperson shall investigate any suspected or alleged non-compliance with this standard, the institutional standard of ethical conduct, or protocol requirements and conditions.

5.2.3.8 No member of the AEC shall be held personally liable for any act committed or omitted by the committee, or member of the committee, in good faith, in the course of the operations of the AEC.

5.2.4 Written proposals

5.2.4.1 Written proposals shall place before the AEC sufficient information to satisfy the AEC that the proposed use of animals is justified and complies with the principles of Replacement, Reduction and Refinement.

5.2.4.2 Written proposals shall be presented in a form that allows the AEC to easily assess the information provided. These shall be written in a manner that can be understood by all members of the AEC and shall identify the impact of all sections of the proposal on animals used and means by which the impact will be minimized.

5.2.4.3 Written proposals shall contain the following information, as appropriate:

- a) the study title;
- b) the names and qualifications of the responsible researchers and all other personnel directly involved, plus a signed statement that the other personnel, including a veterinarian or medical practitioner, named in the protocol have had an opportunity to review the protocol and are familiar with its contents;
- c) an explanation as to the appropriateness of qualifications and past experience in the procedures to be performed and the species to be used;
- d) a clear description in lay terms of
 - 1) the scientific or educational objective of the study, and
 - 2) the expected benefits;
- e) justification of the study which shall address how the study will
 - 1) increase our understanding of humans or animals,
 - 2) maintain or improve human or animal health and welfare,
 - 3) improve animal management or production, or

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- 4) achieve the ecological or educational objectives;
- f) reasons why animals are necessary for the study and, in particular, why techniques which do not use animals have been rejected as unsuitable;
- g) details of what happens to animals from the time they are obtained until the time the study is completed. This should include a description of
 - 1) the study and related procedures, including dose and route of any substances administered,
 - 2) surgical and related procedures, including doses of anaesthetic, analgesic, sedative and tranquillizing agents and methods of monitoring their adequacy,
 - 3) details of the location of the animals' housing or the location of study use (or both),
 - 4) conditions of handling and housing, and
 - 5) arrangements for the disposal of animals at the completion of the study, including methods of euthanasia, if applicable;
- h) identification and justification of all aspects of animal use, including handling and housing, which might impact on an animal's wellbeing, as well as detailed methods to minimize distress or pain, including the monitoring thereof;
- i) details of how animals will be monitored including records of
 - 1) methods and frequency of monitoring both to assess the impact of procedures and to ensure the general wellbeing of an animal on a day-to-day basis,
 - 2) identification and details of welfare indicators that will be used in the monitoring process,
 - 3) personnel involved,
 - 4) details of who will be responsible for the management of emergencies and how it will be ensured that nominees can be contacted, and
 - 5) details of veterinary and para-veterinary supervision;
- j) number and species of animals required, and justification on the basis of study design and statistical considerations;

NOTE The appropriate number of animals (neither too few nor too many) should be used to satisfy statistical requirements.
- k) source of the animals, any necessary permits, and the owner's consent where applicable;
- l) justification for any repetition of previously performed studies;
- m) details and justification of procedures which might cause pain or distress but in which anaesthesia and analgesia cannot be used. The planned endpoint and the reasons for its choice shall be provided and if death as an endpoint cannot be avoided, it shall be justified;
- n) identification of and justification for the use of any animal that has been the subject of a previous scientific study or teaching activity, including details of prior experience;
- o) maximum time individual animals will be held. Protocols shall include expected commencement and completion dates and duration of experiments or studies;

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NOTE Special attention should be given to animals held long-term to ensure adequate monitoring of welfare.

- p) any additional features of the proposal that raise special ethical considerations;
- q) any health risks to other animals or staff, including biohazards. Where required, a Biohazard Safety Compliance Certificate shall be obtained from the IBSC or the Biosafety Officer;
- r) a literature review; and
- s) a declaration signed by the responsible researcher(s) or teacher(s) stating that he or she is currently licensed or authorized to perform scientific studies or teaching activities using animals (if required by legislation), and is aware of responsibilities set out in this standard and in applicable legislation.

5.2.5 Operating procedures

5.2.5.1 AECs shall ensure that operating procedures are established which will enable compliance with the requirements of this standard. Such procedures should cover in particular:

- a) the establishment of a quorum for meetings which shall include at least one member from each Category A, B, C and D (see 5.2.3);

NOTE AEC membership should be such that absenteeism will not result in failure to reach a quorum or lack of balance within the committee.

- b) all matters specific to the institution that will assist compliance with this standard; and
- c) powers that the AEC is prepared to delegate to an Executive Committee.

5.2.5.2 The AEC may establish an Executive Committee which shall include at least one external member from Categories C or D and a staff member (see 5.2.3.2) or his or her deputy. The Executive Committee may approve minor modifications to studies and deal with emergencies, but all decisions by the Executive Committee shall be reviewed by the AEC at its next meeting.

NOTE 1 The AEC might need to put in place procedures to deal with the immediate use of animals for the diagnosis of unexplained and severe disease outbreaks.

NOTE 2 In larger committees, where there are two or more members from Categories C and D, it is preferable that the Executive Committee has representatives from both Categories.

5.2.5.3 The Executive Committee may not approve proposals.

5.2.5.4 Minutes shall be maintained which record decisions and all other aspects of the AEC's operation.

5.2.5.5 Meetings shall be scheduled as frequently as the volume of business demands, but not less than quarterly.

5.2.5.6 The process by which decisions are made shall be fair, consistent and transparent to researchers and teachers, and acceptable to all AEC members.

5.2.5.7 Irreconcilable differences between the AEC and a researcher or teacher shall be referred to the governing body of the institution for review (see 5.1(l)).

5.2.5.8 Standard Operating Procedures (SOPs) that cover all operational studies shall be developed and documents made available for reference and inspections. These SOPs shall be reviewed regularly, updated as required and managed by the person-in-charge (see 3.17).

5.2.6 Assessing proposals

5.2.6.1 Only those scientific studies or teaching activities that conform to the requirements of all relevant clauses of this standard may be approved.

5.2.6.2 Proposals shall be considered and approved only at AEC meetings.

5.2.6.3 Where possible, decisions on the approval of proposals shall be made on the basis of consensus at quorate meetings. Should consensus not be reached, i.e. where two or more members oppose a proposal, the AEC shall explore ways of modifying the study that could lead to consensus. However, should it still be impossible, the decision of the majority shall be accepted.

5.2.6.4 Researchers and teachers shall be informed of decisions in writing.

5.2.6.5 A register of all approved scientific studies and teaching activities shall be maintained.

5.2.6.6 Decisions shall be made as promptly as possible.

5.2.6.7 Scientific studies or teaching activities that involve the use of animals shall not start before written approval is given (see 4.2.5). Failure to obtain such permission shall result in studies not being recognized.

5.2.6.8 Pilot studies (see O.7), where proposed or considered preferable by the AEC, shall be regarded as integral to the overall study or studies. These enable the assessment of the feasibility and value of the study, and the potential for Replacement, Reduction and Refinement.

5.2.7 Monitoring

5.2.7.1 AECs shall ensure that adequate records are kept on the acquisition, breeding, health, care, housing, use and disposal of animals (see also 6.1.10 and 6.3.1.3.1).

5.2.7.2 With due consideration of biosafety requirements, announced and unannounced inspections of all animal housing and laboratory areas shall be conducted regularly by members of the AEC and appropriate records shall be maintained to ensure compliance with this standard. Difficulties might be experienced by AECs in monitoring fieldwork and work in remote locations. AECs shall ensure that adequate records are kept, that appropriate emergency procedures are in place for each study, and that persons involved have appropriate skills and knowledge of current techniques.

5.2.7.3 AECs shall ensure that any activity that constitutes a major breach of this standard (i.e. a breach that has immediate negative implications for animal welfare) ceases immediately and that appropriate action is taken. This may include referral to the person-in-charge of the institution. For non-compliance that has infrastructural dimensions, a reasonable time shall be given for correction, but the AEC shall be assured and kept informed that the problem is being addressed. The AEC shall initiate investigations into any suspected or alleged non-compliance with this standard, institutional policies or the said Animals Protection Act.

5.2.8 Annual review

5.2.8.1 Approved studies of long duration and the long-term continuing use of individual animals shall be reviewed at least annually by the AEC or more frequently if considered desirable. A study can be reviewed if warranted by the emergence of new information (whether scientific or pertaining to the scientific studies or teaching activities or to the researcher).

5.2.8.2 The AEC shall make provisions to audit scientific studies and teaching activities in relation to a researcher's or teacher's compliance with a submitted protocol.

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5.2.9 Reporting to institutions

5.2.9.1 The AEC shall report in writing at least annually to the governing body of the institution on its activities relating to

- a) the number and types of scientific studies and teaching activities approved,
- b) the physical facilities for the care and use of animals within and outside the institution,
- c) administrative or other difficulties being experienced,
- d) any requirements for training of staff,
- e) non-compliance reports, recommendations, actions and outcomes,
- f) categories of research, severity assessments and invasiveness (see 5.2.10),
- g) animal procurement numbers and sources,
- h) numbers, species and sexes of animals supplied and used,
- i) animal waste (overproduction) figures,
- j) monthly census reports,
- k) mortality and morbidity reports,
- l) veterinary reports, including health surveillance issues,
- m) completed and ongoing studies,
- n) Animal Welfare Incident reports,
- o) repairs and maintenance of all animal facilities,
- p) equipment replacement that affects animal welfare, and
- q) funding and resource requirements.

5.2.9.2 All the AEC approved proposals shall be the subject of written reports to the AEC. Regardless of the duration of the approval, the continuation of all activities shall be subject to the receipt of written annual reports that shall advise on

- a) the progress achieved,
- b) the problems that might have interfered with the progress of the study or teaching activity,
- c) the number of animals used to date or in total,
- d) the wellbeing and animal welfare status of all animals during the study or teaching activity,
- e) the unexpected mortalities,
- f) the envisaged modifications, amendments, or additions to the AEC approved proposal,
- g) the possibility of the study or teaching activity in achieving the stated objectives,

- h) the status of the study or teaching activity; whether it is to continue, has been completed, or is discontinued, and
- i) the publications produced, with references.

Following a review of the annual report, the AEC may determine, on the basis of the report and further consultation with the researcher or teacher, that a study or teaching activity may continue, be suspended, require modification or be terminated.

5.2.10 Categorizing proposals

The AEC shall adopt or develop a system to categorize proposals so as to help identify areas of special concern. An example of such an area of special concern is the classification of pain (see 6.3.1.1).

5.2.11 Scientific studies and teaching activities at more than one institution

5.2.11.1 Where scientific studies and teaching activities are to be conducted at more than one institution, AEC approval shall be sought from each institution unless responsibility has been formally delegated to one AEC.

5.2.11.2 When responsibility has been formally delegated to another institution, the researcher shall notify the AEC at his or her own institution, in writing, that there is approval elsewhere for a study.

5.2.12 Non-institutional applicants and the responsibility of the AEC

5.2.12.1 AECs may be approached by individuals or organizations which do not have direct access to an institutional AEC yet require AEC approval before proceeding to use animals for scientific studies and teaching activities.

5.2.12.2 The AEC shall decide, on an individual case basis, whether it is prepared to assess the proposal and oversee the scientific study or teaching activity. In such cases, proposals for non-institutional applicants shall, in addition to all information normally required by the AEC, address

- a) who accepts responsibility for the scientific study or teaching activity, as well as their qualifications, registration and experience,
- b) how the impact of the scientific study or teaching activity on the animals will be monitored, and
- c) the qualifications, registration and experience of co-workers and staff.

5.2.12.3 Arrangements between an institutional AEC and a non-institutional applicant shall be a formally signed agreement between the two. This arrangement shall enable the institution to withdraw from the agreement if the non-institutional applicant fails to comply with the requirements and conditions imposed by the AEC.

6 Responsibilities of researchers and teachers

6.1 General

6.1.1 Researchers and teachers have direct and ultimate ethical and legal responsibility for all matters related to the welfare of animals used. They shall act in accordance with all the requirements of this standard.

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6.1.2 The responsibility of researchers and teachers extends over all facets of the care and use of animals in scientific studies and teaching activities approved by the AEC. This responsibility begins when the animal is sourced and allocated to the approved study or activity, and ends at the time of disposal of the animal.

6.1.3 Researchers and teachers are responsible for the standard of animal care and use by all other persons involved in the study or activity. They shall ensure that the extent of supervision is compatible with the level of competence of each person and the responsibilities they are given.

6.1.4 Researchers and teachers should consult other experienced scientists, veterinarians, or laboratory animal, farm animal or wildlife specialists, when necessary.

6.1.5 Before any scientific study or teaching activity that involves the use of animals can begin, researchers and teachers shall submit a proposal to the AEC that demonstrates that the study will comply with the conditions of this standard and the Animals Protection Act, 1962 (Act No. 71 of 1962).

6.1.6 Researchers and teachers shall not begin a scientific study or teaching activity that involves the use of animals before written AEC approval is obtained (see 4.2.5), and shall adhere to all requirements and conditions imposed by the AEC.

6.1.7 In the event of emergencies, researchers and teachers shall ensure that satisfactory arrangements are made for contacting them and other responsible persons.

6.1.8 Researchers and teachers shall ensure that the choice of species is appropriate for the purpose of the study. Requirements for known genetic constitution, freedom from specific diseases, documented health, nutritional and environmental histories and other relevant factors shall be taken into account. When the definition of the biological status of animals is necessary, researchers and teachers shall ensure that the supplier can provide adequate proof of definition. Where relevant, species and individual animals should be chosen on the basis that the proposed studies will result in the least pain and distress. In making this decision, all aspects of the biological nature of the animals, including their behavioural characteristics and their cognitive abilities and development, shall be taken into account.

6.1.9 Researchers and teachers shall ensure that all intensively managed animals are observed daily (or more frequently if circumstances require it) in order to assess their health and welfare.

6.1.10 Researchers and teachers shall ensure that records of the use and monitoring of animals in scientific studies and teaching activities are maintained.

6.1.11 Researchers and teachers shall inform the AEC when an approved scientific study or teaching activity is completed or discontinued.

6.1.12 In the ethical application form, it shall be explained to the AEC what is generally going to be done in case of unexpected events that impact on animal welfare (for example, immediately stopping treatment and seeking veterinary advice). In longer term studies, scientists shall furnish progress reports to the AEC twice yearly.

6.1.13 When conducting procedures using animals, researchers and teachers shall comply with the regulatory requirements of the Veterinary and Para-Veterinary Professions relating to Acts which are deemed to pertain specially to the veterinary profession.

6.2 Planning studies

In addition to the information required by the AEC, the researchers and teachers need to address the following questions during the planning stages of a scientific study or teaching activity:

- a) Is the scientific study or teaching activity ethically and scientifically justified?
- b) Can the aims be achieved without the use of animals?
- c) Has the most appropriate species of animal been selected?
- d) Are suitable holding facilities available?
- e) Are suitable, competent staff available?
- f) Have all staff been informed of the planned study and other procedures?
- g) Is the biological status (genetic, nutritional, microbiological and general health) of the animals appropriate?
- h) Are the environmental conditions (including caging or pen type, noise, photoperiod, temperature, humidity, ventilation, density of housing, social structures and environmental enrichment) appropriate?
- i) Are the scientific studies designed so that statistically valid results can be obtained or the educational objectives achieved using the minimum necessary number of animals?
- j) If the scientific study or teaching activity could cause the animals any pain or distress, what will be done to minimize or avoid this?
- k) What arrangements will be made to monitor the animals adequately?
- l) Have provisions been made for records to include details of animal husbandry routine, environmental conditions, and other potential non-study variables which might affect the study?
- m) Have any of the scientific studies or teaching activities been performed previously? If so, why should they be repeated?
- n) Are any official permits required for the importation, capture, use, disposal or release of the animals? and
- o) Are checklists and guidelines for the correct completion of research protocols by researchers used?

NOTE Where the AEC deems it necessary, it may request documentary backup to the answers given to the questions in 6.2 (a) to (g).

6.3 Conduct of scientific studies and teaching activities

6.3.1 General considerations

6.3.1.1 Limiting pain and distress

6.3.1.1.1 Pain and distress cannot be evaluated easily in animals, therefore, researchers and teachers shall assume that animals experience pain in a manner similar to humans. Decisions

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regarding their welfare in scientific studies and teaching activities shall be based on this assumption unless there is evidence to the contrary.

6.3.1.1.2 Researchers and teachers shall anticipate and take all possible steps to avoid or minimize pain and distress, including:

- a) choosing the most appropriate and humane method for the conduct of the scientific study or teaching activity;
- b) ensuring that the technical skills and competence of all persons involved in animal care and use are adequate for the tasks that are performed;
- c) ensuring that animals are adequately monitored for evidence of pain and distress;
- d) acting promptly to alleviate pain or distress;
- e) using anaesthetic, analgesic, sedative and tranquillizing agents appropriate to the species and the scientific or educational aims (see 5.1(b));
- f) conducting studies over the shortest time practicable; and
- g) using appropriate methods of euthanasia.

6.3.1.1.3 The use of local or general anaesthetic, analgesic, sedative or tranquillizing agents shall be appropriate to the species, and shall at least equate to their use in current medical or veterinary practice.

6.3.1.1.4 Scientific studies and teaching activities which are liable to cause pain of a kind and degree for which anaesthesia would normally be used in medical or veterinary practice shall be carried out under anaesthesia.

6.3.1.1.5 Distress can sometimes be avoided or minimized by non-pharmacological means. Before a scientific study or teaching activity begins, animals shall be appropriately conditioned to the study environment and procedures, and be familiar with handlers. During and after study procedures, appropriate nursing to minimize pain and distress, and to promote the wellbeing of the animals, shall be provided.

6.3.1.1.6 The monitoring of animals shall at all times be adequate to prevent the occurrence, or allow prompt alleviation, of pain or distress.

6.3.1.1.7 If animals develop signs of severe pain or distress, despite the precautions outlined in 6.3.1.1.1 to 6.3.1.1.6, then they shall have the pain or distress alleviated promptly or shall be put to death by recognized euthanasia methods, without delay. Alleviation of such pain or distress shall take precedence over continuing or finishing the study. If in doubt, then researchers and teachers shall always seek professional veterinary opinion before continuing a study.

6.3.1.2 Signs of pain or distress

6.3.1.2.1 Researchers and teachers shall be familiar with the normal behaviour and husbandry of the animal species chosen, shall be knowledgeable of signs of pain and distress specific to that species, and shall be responsible for the monitoring of animals for these signs.

6.3.1.2.2 Animals shall be monitored to allow detection of deviations from expected behaviour patterns for the species. Such deviations are often the first indications that animals are experiencing pain or distress. Assessments of change in patterns of sleeping, feeding, drinking, grooming,

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exploratory behaviour, exercise, performance in learning or discriminatory tasks, reproduction or social behaviour shall be made.

6.3.1.2.3 Animals shall be monitored appropriately for clinical signs of pain or distress. These signs might include aggressive or abnormal behaviour (or both) (some species might become unduly submissive), abnormal stance or movements, abnormal sounds, altered cardiovascular or respiratory function (or both), abnormal appetite, rapid decline in bodyweight, altered body temperature, vomiting and abnormal defecation or urination. Indicators of sustained pain or distress might include loss of body weight, failure to thrive, impaired reproductive ability and reduced resistance to disease.

6.3.1.3 Repeated use of animals in scientific studies and teaching activities

6.3.1.3.1 Individual animals shall not be used in more than one study or teaching activity either in the same or different studies or teaching activities, without the expressed approval of the AEC. However, appropriate reuse of animals might reduce the total number of animals used in a study or teaching activity, result in better study design, reduce distress or avoid pain to other animals.

6.3.1.3.2 When approving studies that involve the reuse of animals, the AEC shall be satisfied that no animal which has been used in a procedure entailing severe pain or enduring pain or suffering, irrespective of whether anaesthesia or analgesia was employed, shall be used in a further procedure unless it has returned to good health and wellbeing. Approval for reuse should take into account the housing conditions, given that this will result in a longer period of housing.

6.3.1.4 Duration of scientific studies and teaching activities

Scientific studies and teaching activities, particularly those that involve pain or distress, shall be as brief as practicable. The expected duration of the scientific activity shall be clearly defined and provided to the AEC. Should the activity be longer than anticipated, approval from the AEC for continued use shall be sought.

6.3.1.5 Handling and restraining animals

6.3.1.5.1 Animals shall be handled only by persons instructed and competent in methods that avoid distress and do not cause injury.

6.3.1.5.2 The use of restraining devices is sometimes necessary for the welfare of the animal and the safety of the handler. Restraint devices shall be humane, shall be used to the minimum extent, and shall be appropriate for the animal for the minimum period required to accomplish the purpose of the study.

6.3.1.5.3 Tranquillizers, sedatives or anaesthetics might aid restraint but might also prolong recovery from the procedure. When these agents have been used, the recovery of the animals shall be monitored. Researchers and teachers shall be cognitive of the paradoxical effects that certain tranquillizers and sedatives can exercise on the behaviour of the recovering animal (excitement, delirium, aggressiveness, etc.) and shall therefore take preventative measures such as the use of suitable antagonists. This will prevent the animal from hurting itself or the researchers and teachers.

6.3.1.5.4 Periods of prolonged restraint shall be approved by the AEC. Where animals are in prolonged restraint, consideration shall be given to their biological needs, including their behavioural requirements, and they shall be monitored regularly by a veterinarian or a laboratory animal technologist not participating in the study. If any ill effects are shown, the animal shall be removed from the restraint or the method shall be modified.

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6.3.1.6 Completion of scientific studies and teaching activities

6.3.1.6.1 Upon completion of the scientific study or teaching activity, animals shall be returned promptly to either normal husbandry conditions or, if appropriate and permitted, to their natural habitat, or be humanely put to death by recognized euthanasia methods.

6.3.1.6.2 Where practicable, researchers and teachers shall share tissue from animals being put to death by recognized euthanasia methods with other researchers and teachers.

6.3.1.6.3 For scientific studies or teaching activities that have been completed or discontinued, a report shall be submitted to the AEC as soon as practicable. This report shall advise on

- a) whether the stated aims and objectives were achieved,
- b) the total number of animals used and any discrepancies or unexpected issues,
- c) whether there were any animal welfare issues,
- d) the reasons for the discontinuation of the study or teaching activity,
- e) recommendations for future modifications and improvements to the study or teaching activity,
- f) recommendations for reduction and refinement of animal use, and
- g) details of publications resultant from the study or teaching activity, with references.

6.3.1.7 Euthanasia

6.3.1.7.1 When it is necessary to kill an animal, humane procedures shall be used. These procedures shall avoid distress, shall be reliable, and shall produce rapid loss of consciousness without pain until death occurs. The procedures shall also be compatible with the scientific or educational aims.

6.3.1.7.2 The procedures shall be performed only by persons competent in the methods to be used, or under the direct supervision of a competent person. The appropriate means shall be readily at hand.

6.3.1.7.3 Animals shall be put to death by recognized euthanasia methods in a quiet, clean environment, and away from other animals unless defensible reasons, approved by the AEC, dictate otherwise. There shall be no disposal of an animal's body until death is established.

6.3.1.7.4 Dependent neonates of animals being killed shall also be put to death by recognized euthanasia methods or provision shall be made for their care.

6.3.1.7.5 When fertilized eggs are used, the method of disposal shall ensure the death of the embryo.

6.3.1.8 Autopsy

An autopsy shall be performed by a competent and qualified person when animals in housing and breeding programs die or are humanely put to death due to illness. A facility for postmortem examinations and for safe disposal procedures shall be provided separate from facilities used for the housing of animals and experimental procedures.

6.3.1.9 Serious adverse events (including unexpected death)

The researcher or teacher shall report any serious adverse events, including unexpected deaths, to the AEC, the person-in-charge and, if applicable, to the relevant regulatory authorities. In the cases of pre-clinical and clinical safety studies, for instance, the Medicines Control Council shall be notified of any serious adverse events. The respective time frames for the reporting of such events shall be stipulated by the AEC, the person-in-charge or in accordance with the relevant regulatory authority as stated in the Agricultural Stock Remedies Act, 1947 (Act No. 36 of 1947) and the Related Substances Act, 1965 (Act No. 101 of 1965), as amended, where applicable.

6.3.1.10 Endpoint evaluations

6.3.1.10.1 When preparing a study application, for all but the most minor manipulations, the researchers and teachers shall develop humane study endpoints which can be used to judge when an animal requires to be put to death by recognized euthanasia methods for animal welfare reasons.

6.3.1.10.2 Death as an endpoint is generally ethically unacceptable and shall be fully justified. All animals found in a moribund state shall be put to death by recognized euthanasia methods since these animals give unreliable research data due to multiple organ failure. Endpoints earlier than a moribund condition shall always be used.

6.3.2 Additional considerations

6.3.2.1 Surgery

6.3.2.1.1 Anaesthesia and surgery shall be performed by competent professional staff with appropriate training and experience. Instruction in surgical or anaesthetic techniques shall be under the direct and constant supervision of such persons. Adequate knowledge of topics such as animal physiology, pharmacology and anatomy is essential for the success of any research programme that involves the use of laboratory animals, especially where surgical techniques are required.

6.3.2.1.2 Surgical procedures shall be carried out under appropriate local or general anaesthesia. There shall be adequate monitoring of the depth of anaesthesia and of side effects such as hypothermia, and cardiovascular and respiratory depression.

6.3.2.1.3 When more than one surgical procedure is to be performed, the animal shall have recovered to good general health between each procedure. Every effort shall be made to reduce the total number of procedures and the AEC shall have been informed specifically of the need for more than one.

6.3.2.1.4 When the animal is not to recover from the surgery, it shall be rendered unconscious for the whole procedure, either by continuing the administration of the general anaesthetic or by euthanasia.

6.3.2.1.5 When the animal is to recover from the anaesthetic, surgical procedures shall conform to accepted standards in human and veterinary practice.

6.3.2.1.6 The aseptic technique shall be used for animals that undergo major survival surgery. This is defined as any surgical intervention that penetrates a body cavity or has the potential for producing a permanent handicap in an animal that is expected to recover. This technique involves aseptic preparation of the surgical field, use of sterilized instruments, and wearing of sterile surgical gloves, gowns, caps, and face masks. The use of post-operative antibiotics shall not be a substitute for correct aseptic technique.

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6.3.2.2 Post-operative care

6.3.2.2.1 The comfort of animals shall be promoted throughout the post-operative period. Attention shall be given to warmth, hygiene, fluid and food intake, and control of infection. Analgesics, sedatives and tranquillizers shall be used to minimize post-operative pain or distress. Care shall be taken that animals recovering from anaesthesia do not injure themselves by unco-ordinated movements, and that conditions are such that they are not disturbed, attacked or killed by other animals in the same enclosure.

6.3.2.2.2 Appropriate clinical records shall be kept and made accessible to all involved in the post-operative care of the animal.

6.3.2.2.3 Researchers and teachers shall ensure that adequate monitoring, treatment and care of post-operative animals is provided. They shall ensure that they are fully informed of the animal's condition by designated competent staff.

6.3.2.2.4 The duties of all staff shall be clearly defined and ways of dealing with emergencies established.

6.3.2.2.5 Any post-operative animal observed to be in a state of severe pain or distress which cannot be alleviated quickly shall be put to death by recognized euthanasia methods without delay.

6.3.2.2.6 Regular observation of surgical wounds is essential to check the progress of healing. Any problems shall be attended to immediately.

6.3.2.3 Implanted devices

6.3.2.3.1 Skilled and specialized attention is required in the care of animals following an operation in which monitoring or sampling devices have been implanted, or a fistula created. Regular observation is essential to determine signs of distress, pain or infection, which shall be treated immediately.

6.3.2.3.2 Researchers and teachers shall be aware of the need for strict attention to aseptic technique when foreign bodies are surgically implanted. Contamination of prosthetic devices frequently requires their removal after antibiotic therapy has failed.

6.3.2.4 Neuromuscular paralysis

Neuromuscular blocking agents shall never be used for immobilization without adequate general anaesthesia or an appropriate surgical procedure that eliminates sensory awareness. Immobilization of an animal solely with a neuromuscular blocking agent is not acceptable. When these agents are used with an anaesthetic, special care shall be taken to ensure the maintenance of an adequate plane of anaesthesia. Since criteria such as the character of respiration and corneal and flexor withdrawal reflexes cannot be used, continuous or frequent intermittent monitoring of physiological variables such as heart rate, blood pressure, pupil size and the electroencephalogram is necessary, together with the effects of these on mild sensory stimuli. Care is required to ensure that drugs used during procedures do not interfere with this monitoring.

6.3.2.5 Electroimmobilization

Electroimmobilization shall never be used as an alternative to analgesia or anaesthesia.

6.3.2.6 Animal models of disease

The scientific validity of animal models of human diseases rests in part on how closely they resemble a particular disease. Thus the attendant pain and distress of the human diseases might

also occur in an animal. Special care shall be taken in selecting the appropriate species and the researcher or teacher shall accept responsibility for ensuring that any pain or distress is minimized and that the AEC is informed of the potential effects of the disease on the animals.

6.3.2.7 Modifying animal behaviour

The preferred inducement is positive reinforcement. However, the inducement might be some form of biological stress, but this stress shall be as mild as possible. Severe water, food, social or sensory deprivation shall not be used to modify behaviour. Behaviour can usually be modified using procedures that involve no more than a physiological stress (for example, thirst within the range of the normal experience of the species). When stimuli are used to modify behaviour, the AEC shall be aware of the duration and an escape route from the stimuli shall be provided where possible.

6.3.2.8 Toxicological studies

6.3.2.8.1 Investigation of the safety of chemicals, medical substances or preparations, devices and naturally occurring toxins intended for use by human beings, animals, the household or the environment, shall be performed by persons with appropriate training. If suitable non-animal tests are available, they shall be used. In particular, *in vitro* methods should be used as an initial screening test wherever possible.

6.3.2.8.2 The endpoint of such studies shall be determined as early as is compatible with reliable assessment of toxicity, and shall minimize the extent of any pain and distress.

6.3.2.8.3 Researchers shall not allow scientific studies to proceed to the point of painful, distressful or lingering death of animals.

6.3.2.8.4 When death as an endpoint cannot be avoided, the studies shall be expressly justified to the AEC before use and shall be designed to result in the deaths of as few animals as possible so as to meet scientific and statistical requirements.

6.3.2.9 Scientific studies and teaching activities that involve hazards to humans or other animals

6.3.2.9.1 Hazards might arise from sources such as viruses, bacteria, fungi, parasites, radiation, radioactivity, corrosive substances, toxins, allergens, carcinogens, recombinant DNA, anaesthetic gases and physical injuries.

6.3.2.9.2 Any potential pathogenic effects of these hazards, when used in scientific studies or teaching activities, shall be explained as far as possible to all staff. Tests to detect possible acquired pathogens might be required for staff during and after the scientific study or teaching activity.

6.3.2.9.3 The AEC shall check that the advice of the institution's biohazards committee has been sought and that appropriate measures for containment, disposal and decontamination have been established.

6.3.2.9.4 Animals being administered infectious organisms shall be quarantined or isolated, as appropriate, taking into account the risks to other animals and to humans.

6.3.2.9.5 Protocols submitted to the institution's AEC shall include a description of any intended use of hazardous compounds or organisms. They shall describe specific safety measures and disposal protocols used to prevent contamination of caging of other animals, of research personnel and students, and of the environment. All researchers and teachers involved, as well as administrators of facilities accommodating research with infectious agents shall adhere to the recommendations of the Centers for Disease Control and Prevention, Atlanta, USA.

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6.3.2.9.6 The endpoint of scientific studies or teaching activities that involve hazardous agents shall conform to the requirements for toxicological studies (see 6.3.2.8).

6.3.2.9.7 Precautions, security and emergency plans to contain hazardous agents shall be appropriate to a "worst-case" situation.

6.3.2.9.8 Institutions, AECs and animal research facilities shall have developed and documented procedures and management plans for emergency situations and for the management of bio-hazardous substances, and shall have appointed and designated personnel to deal with such contingencies. The following are important considerations

- a) Fire and flood.
- b) Equipment failure.
- c) Emergency power supply.
- d) Contact persons and their telephone numbers in emergencies.
- e) Emergency services, i.e. fire brigade, ambulance service, electricians and plumbers.
- f) Building evacuation procedures, including evacuation of animals.
- g) Equipment safety, servicing and compliance certification.
- h) Life support systems.
- i) First-aid facilities.
- j) Firefighting equipment.
- k) Emergency exits.
- l) Spill containment.
- m) Protective clothing and showers.
- n) Chemicals, drugs and biohazardous substances, including their storage and containment.
- o) Security control measures.

6.3.2.10 Animal welfare and animal health research

When studying ways of improving the health or welfare of animals, researchers might need to design studies that replicate the problem such as injury, trauma, nutritional disorder, physical exertion, disease or environmental stress so that the attendant pain or distress might also be replicated. When such studies are necessary, the researcher shall ensure that:

- a) the principal aim of the study is to improve animal welfare or health;
- b) alternative methods are not possible such as the use of animals already showing symptoms related to the specific affliction to be investigated;
- c) all possible steps are taken to minimize any pain or distress; and
- d) the endpoints of studies conform to the requirements for toxicological studies (see 6.3.2.8).

6.3.2.11 Study manipulation of animals' genetic material

6.3.2.11.1 Work that involves the introduction of foreign DNA into mammalian cells or whole animals shall be conducted in accordance with guidelines issued by the South African Committee for Genetic Experimentation and the relevant biohazards committee of the institution.

6.3.2.11.2 All proposals to manipulate the genetic material of animals, their germ cells or embryos shall also be submitted to an AEC for approval.

6.3.2.11.3 The manipulation of the genetic material of animals has the potential to affect the welfare of the animals and their offspring adversely. Researchers shall inform the AEC of the known potential adverse effects on the wellbeing of the animals.

6.3.2.11.4 The clinical status of animals in which the genetic material has been manipulated shall be monitored for unusual or unexpected adverse effects. Researchers shall report such effects to the AEC and appropriate action shall be taken, including euthanasia if necessary.

6.3.2.12 Study induction of neoplasia

6.3.2.12.1 The site for induction of tumours (neoplasia) shall be chosen carefully. Subcutaneous, intradermal and flank sites shall be chosen when possible. Footpad, brain and eye sites shall not be chosen unless specifically justified to the AEC before use.

6.3.2.12.2 Researchers shall monitor animals in their care closely for signs of pain or distress, especially sudden changes in body weight.

6.3.2.12.3 Animals with induced tumours shall be killed humanely before predictable death occurs, and before cachexia becomes advanced or the tumour becomes large enough to cause ulceration or severe limiting of normal behaviour.

6.3.2.12.4 With ascitic tumours, including hybridomas, researchers shall ensure that the volume of ascitic fluid does not cause gross abdominal distension, and the volumes of solid tumours and cachexia do not become distressful to the animals.

6.3.2.12.5 In tumour therapy studies, the endpoints chosen shall be as early as possible, compatible with reliable assessment of the therapy. Weight changes shall be monitored closely. Death from the tumour shall not be chosen as a study endpoint unless in accordance with 6.3.2.8 and with the approval of the AEC.

6.3.2.13 Lesions of the central nervous system

Studies of anatomical or chemical lesions of the central nervous system demand special consideration when the lesion results in loss or impairment of limb or trunk movements, loss of sensibility to touch, change in temperature, pain, and impairment of the animal's awareness of its surroundings or impairment of appetite or thirst mechanisms. Special animal care, caging, and other facilities might be needed, and the AEC, in approving such studies, has a particular responsibility to ensure that these facilities are available and that the condition of the animal is closely monitored.

6.3.2.14 Withholding food or water

Studies that involve the withholding or severe restriction of food or water shall produce no continuing detrimental effect on the animal. In these studies, the fluid balance or body weight (or both) shall be monitored, recorded and maintained within the limits approved by the AEC.

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6.3.2.15 Foetal experimentation

6.3.2.15.1 When foetal experimentation or surgery compromises the ability of the neonate to survive without pain or distress, the neonate shall be killed humanely before or immediately following birth unless such pain or distress can be relieved.

6.3.2.15.2 Unless there is specific evidence to the contrary, researchers shall assume foetuses have the same requirements for anaesthesia and analgesia as adult animals of the species.

6.3.2.15.3 During surgery of the mother, consideration shall be given to any special requirements for anaesthesia of the foetus.

6.3.2.15.4 Eggs shall be destroyed before hatching, unless hatching is a requirement of the study. The AEC shall approve the arrangements made for the hatchlings.

6.3.2.16 Research on pain mechanisms and the relief of pain

In studies in which unanaesthetised animals are to be subjected to stimuli designed to produce pain, researchers shall:

- a) ensure that these stimuli limit pain at all times to levels comparable to those which do not distress human beings;
- b) ensure that the animals are exposed to the minimum pain necessary for the purpose of the procedure; and
- c) provide treatment for the relief of pain, allow self-administration of analgesics, or escape from repetitive, painful stimuli, when possible.

7 Acquisition and care of animals in breeding and holding areas

7.1 General

7.1.1 There are a number of requirements governing the import, capture, handling and transport of animals obtained from other countries.

7.1.2 Transport can cause distress to animals due to confinement, movement, noise and changes in the environment and personnel.

7.1.3 The extent of any distress will depend on the animals' health, temperament, species, age, sex, the number travelling together and their social relationships, the period without food or water, the duration, the mode of transport, environmental conditions particularly extremes of temperature, and the care given during the journey.

7.1.4 The conditions and duration of the transport shall ensure that the health and wellbeing of the animals is not unduly compromised.

7.1.5 Potential sources of distress shall be identified and steps taken to avoid or minimize their effects on the animals.

7.1.6 Containers shall be escape-proof and tamper-proof, there shall be adequate nesting or bedding material, and animals shall be protected from sudden movements and extremes in ambient temperatures.

7.1.7 Food and water shall be provided when necessary.

7.1.8 Local transport of livestock shall comply with the standard for the Transport of Livestock published by the Livestock Welfare Co-ordinating Committee¹⁾ and, where applicable, the requirements for the transport of wild herbivores in SANS 10331.

7.1.9 Both the suppliers and recipients of animals shall ensure that there are satisfactory delivery procedures, and that animals are received by a responsible person.

7.1.10 All institutions, AECs, animal research facilities and persons involved with the transport of animals for research purposes shall have documented SOPs describing these activities. These procedures shall include emergency procedures, amongst others, for recumbent animals and for animal escape. Equipment to be considered for inclusion in the SOPs shall include:

- a) a cell phone;
- b) an emergency drugs kit;
- c) a backup vehicle;
- d) compartment ventilation and temperature control mechanisms; and
- e) firearms and a captive bolt.

7.2 Animals obtained from facilities within the Republic of South Africa (RSA)

7.2.1 Animals shall be obtained from breeding and supply facilities that maintain conditions and uphold standards consistent with this standard, relevant legislation or relevant industry standards.

7.2.2 Animals which are for use in scientific studies and teaching activities, and of the species listed in table 1, shall be acquired directly from or originate from a breeding establishment, unless a general or special exemption has been obtained under arrangements to be determined by the AEC.

Table 1 — Animals for use in scientific studies and teaching activities

1	2
Common name	Latin name
Mouse	<i>Mus musculus</i>
Rat	<i>Rattus norvegicus</i>
Guinea pig	<i>Cavia porcellus</i>
Hamster	<i>Mesocricetus auratus</i>
Rabbit	<i>Oryctolagus cuniculus</i>
Dog	<i>Canis familiaris</i>
Cat	<i>Felis catus</i>

7.2.3 Institutions shall undertake to extend the provisions of 7.2.1 to other species, in particular primates, as soon as there is a reasonable prospect of a sufficient supply of purpose-bred animals of the species concerned.

1) Obtainable at www.samic.co.za.

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7.2.4 Stray animals of a domesticated species shall not be used in scientific studies and teaching activities. A general exemption made under the conditions of 7.2.1 may not extend to stray dogs and cats (also see annex F).

7.2.5 Permits to trap, hold, transport and import or export indigenous species between provinces in the RSA shall also be obtained from the various nature conservation authorities.

7.3 Animals obtained from other countries

7.3.1 Animal imports will only be allowed if the importer is in possession of the necessary import permits and veterinary health certificates from the Department of Agriculture, the Chief Directorate Veterinary Services and Livestock Improvement, and the Department of Animal Health.

7.3.2 Crates for the transport of all domestic and wild animals by air shall comply with the International Air Transport Association (IATA) Live Animal Regulations for air transport and the requirements specified by the relevant provincial nature conservation authority. The welfare and safety of the animals on the ground and in the air are of utmost importance and all persons involved with the air freighting shall be familiar with the specific crating and care of the animals to ensure that the animals arrive at their destination in good condition and health.

7.4 Admission of new animals into holding facilities

7.4.1 When new animals are being admitted into animal holding facilities, they shall be accompanied by correct health screening records and veterinary declarations from the exporting facility and country. They shall be correctly identified, and be quarantined or isolated for the appropriate period to allow for their acclimatization to the holding facility and staff.

The animals shall be inspected by a person registered and authorized by the South African Veterinary Council. Their health shall be evaluated, treatment instigated if required, and their suitability for the proposed studies and activities assessed.

7.4.2 Animals that do not adapt satisfactorily to their new environment shall not be kept. Means of disposal will depend on the circumstances (for example, whether they were custom-bred or wild-caught), will honour any existing agreements between supplier and end-user, and will adhere to the provisions of any relevant permits issued. They might thus be returned to the supplier, be re-allocated to another study (acute) if there is one, or be put to death by recognized euthanasia methods and thereafter disposed of.

7.5 Care of animals in holding and production facilities

7.5.1 General

7.5.1.1 Facilities include the buildings, cages (including isolation cages for sick animals), pens, stalls or pastures in which animals are kept.

7.5.1.2 Institutions, researchers, teachers and AECs shall ensure that facilities are appropriately staffed, designed, constructed, equipped and maintained to achieve a high standard of animal care and fulfil scientific requirements.

7.5.1.3 The design and management of facilities will depend on the type of animals to be kept and the studies and activities to be undertaken. The overall condition and management of facilities shall permit effective maintenance and servicing and shall be compatible with the maintenance of animals in good health.

7.5.1.4 The following support areas require described management SOPs:

- ablutions for staff within animal areas;
- animal issues (for example, husbandry, care and procedures);
- animal procedure room(s);
- animal receiving;
- aseptic surgery;
- autoclaving and sterilization;
- biohazard waste storage and disposal;
- cage wash and sanitizing;
- disinfection rooms or locks;
- dirty bedding and waste disposal;
- food store;
- isolation (animal biosafety level (ABSL) 1 to 4);
- kitchen facilities within animal areas;
- postmortem facility;
- quarantine;
- refrigeration and cold room; and
- vehicle wash and sanitizing;

7.5.1.5 The following support areas need to be considered in a documented management plan:

- administration offices;
- animal procedure room(s);
- bedding store;
- clean cage store;
- chemical and flammables store (also covered by the Occupational Health and Safety Act, 1993 (Act No. 85 of 1993));
- consumables store;
- dining or lounge area for staff;
- equipment store;
- laboratory facilities;
- laundry;
- mechanical and plant rooms;
- records room;
- service corridors; and
- X-rays or imaging (also covered by the Occupational Health and Safety Act, 1993 (Act No. 85 of 1993), the Health Professions Act, 1974 (Act No. 56 of 1974) and the Code of Practice of Industrial Radiography – X-Ray Radiography, 2007/01).

7.5.2 Outdoor holding facilities

Outdoor holding facilities shall be compatible with the needs of the species, shall provide adequate shelter, food and water, shall protect the animals from predation and other dangers, and shall meet other species-specific needs. Outdoor housing is associated with greater risk to disease, injury and bullying by conspecifics. Vigilant health monitoring will be required.

7.5.3 Indoor housing (see annexes B to N)

7.5.3.1 Buildings shall provide for the needs of the animals to be housed, and the studies undertaken. Facilities for free movement and group contact are especially important for some species of animals.

7.5.3.2 Buildings shall be designed, operated and maintained to control environmental factors appropriately, to exclude pest animals and to limit contamination associated with the keeping of animals, the delivery of food, water and bedding, and the entry of people and other animals.

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7.5.3.3 Buildings shall be maintained in good repair. Walls and floors shall be constructed of durable materials that can be cleaned and disinfected readily.

7.5.3.4 Buildings shall be kept clean and tidy, and operated to achieve the effective control of pest animals. A pest control programme that covers all areas shall be in place.

7.5.3.5 Adequate separate storage facilities shall be provided for feed, bedding, chemicals, clean cages, drugs, equipment and records, etc.

7.5.3.6 Detergents, disinfectants, deodorants and pesticides might contaminate the animals' environment and choice of agents shall be made in consultation with researchers.

7.5.3.7 There shall be a reticulated water supply and proper facilities for drainage, if appropriate.

7.5.3.8 There shall be adequate contingency plans to cover emergencies such as the breakdown of lighting, heating or cooling, and fire or flood.

7.5.3.9 Precautions shall be taken against the entry of unauthorized persons or products (or both).

7.5.3.10 There shall be a separate storage area for flammables that is lockable and secure with access control, and that complies with the relevant municipal by-laws and the Occupational Health and Safety Act, 1993 (Act No. 85 of 1993).

7.5.3.11 Deodorants or air-fresheners designed to mask unfavourable odours shall not to be used. Deodorants and air-fresheners shall not be substitutes for poor cage hygiene or deficient ventilation.

7.5.3.12 Flow paths describing movements of animals, personnel, feed, bedding, wastes, etc., are recommended to obtain a separation and non-mixing of studies and products. Where possible, the flow shall be from clean to dirty areas in a one-way direction.

7.5.4 Environmental factors

7.5.4.1 Animals shall be provided with environmental conditions that suit their biological needs unless otherwise approved by the AEC for the purposes of a study.

7.5.4.2 Air exchange, temperature, humidity, light, noise and vibration shall be maintained within limits compatible with the health and wellbeing of the animals.

NOTE Satisfactory and effective ventilation is essential for the comfort of animals and for the control of temperature, humidity, and odours. Ventilation systems should distribute air uniformly and achieve adequate air exchange.

7.5.4.3 Noxious odours, particularly ammonia, shall be kept to a level compatible with the health and comfort of the animals and staff. The adequacy of the ventilation system, the design, construction and placement of cages and containers, population densities both within cages and within a room, the effectiveness of the cleaning and the frequency of bedding changes, will all influence the level of noxious gases. Attention shall be given to the balance between the need for cleanliness and the potential impact of cleaning procedures on the animals. Deodorants or air-fresheners shall not be used to mask noxious odours.

7.5.4.4 The environmental factors in 7.5.4.2 and 7.5.4.3 could affect the welfare of the animals and the results of scientific studies and teaching activities. Researchers shall be informed in advance of planned changes to the environmental conditions of animals in their care.

7.5.4.5 Animals shall be housed away from noisy areas and machinery that emit loud noises and low frequency vibrations. Noise producing machinery shall be sited as far away from animal housing as possible.

NOTE Sudden loud noises should be avoided.

7.5.4.6 Noise produced by animals and animal care personnel shall be considered in animal facility designs and operation. Factors such as intensity, frequency, rapidity of onset, duration, and vibration potential of the sound(s), as well as the hearing range and sound susceptibility of the species or strain shall also be considered.

When designing animal housing, suitable materials that do not compromise hygiene and cleaning requirements shall be used to minimize noise levels.

7.5.4.7 Staff shall be trained to avoid practices that generate excessive and unnecessary noise.

NOTE Separation of human and animal areas will minimize disturbances.

7.5.4.8 Procedures, or manipulations on animals, shall be conducted in separate designated areas, well away from other animals.

7.5.5 Food and water

7.5.5.1 Animals shall receive appropriate, uncontaminated and nutritionally adequate food in accordance with acceptable requirements for the species. The food shall be in sufficient quantity and of appropriate composition to maintain normal growth, health and wellbeing of immature animals or normal health and wellbeing of adult animals and the requirements of pregnancy or lactation. Uneaten perishable food shall be removed promptly unless contrary to the needs of the species.

Food shall be prepared in a separate, hygienic, animal diet kitchen or a designated area.

7.5.5.2 The manner in which food and water is provided is important as it might vary in accordance with the species and shall be such as to satisfy the physiological and behavioural needs of the animal.

7.5.5.3 When animals are fed in groups, there shall be sufficient trough space or feeding points to avoid undue competition for food, especially if feed is restricted. Feeding space shall be determined by the size and number of animals that shall eat at one time. Care shall be taken to ensure that subordinate animals have adequate access to food and water.

7.5.5.4 Where the withholding of food is necessary for scientific or safety reasons, such as before anaesthesia, care shall be taken that animals deprived of food are not stressed by exclusion from food whilst other animals around them are fed. This might necessitate removal to another cage or room.

7.5.5.5 Uncontaminated potable drinking water in suitable containers shall always be available.

7.5.5.6 Where required, food shall be stored in accordance with the manufacturer's recommendations. Dry foods shall be kept in a cool, vermin-free storage area. Perishable foods (meats, fruits and vegetables) shall be refrigerated.

NOTE 1 Refrigeration preserves nutritional quality and lengthens shelf life.

NOTE 2 Exposure to temperatures above 21 °C, high relative humidity, unsanitary conditions, light, oxygen and vermin will hasten the deterioration of food.

7.5.5.7 Variations in the requirements of 7.5.5.1 to 7.5.5.4 (inclusive) as part of a study design shall receive prior AEC approval.

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7.5.6 Pens, cages, containers and the immediate environment of the animals (see annexes B to N)

7.5.6.1 Animal accommodation shall be designed and managed to meet species-specific needs. Pens, cages and containers shall be constructed and maintained to ensure the comfort and wellbeing of the animals. The following factors shall be taken into account:

- a) the species-specific behavioural needs, including the availability and design of space to enable free movement and normal activity, sleeping, privacy and behavioural security, and contact with others of the same species;
- b) the provision of single housing for animals when it is appropriate for the species and if necessary for the purpose of the study as approved by the AEC (for example, during recovery from surgery or during collection of samples);
- c) the species-specific environmental requirements such as lighting, temperature, air quality, appropriate day/night cycles and protection from excessive noise and vibrations. Care shall be taken that apparatus, such as pumps that might emit noise in the ultrasonic range, are not installed near or in animal quarters;
- d) the need to provide ready access to food and water;
- e) the need to clean the pen, cage or container regularly and routinely, with attention to contact bedding, non-contact bedding and safe waste disposal procedures and procedures for assessing the effectiveness of the sanitizing programme;
- f) the protection from the spread of pests and disease;
- g) the requirements of the scientific study or teaching activity;
- h) the need to observe the animals readily; and
- i) the passages' cleaning and sanitizing programme.

7.5.6.2 Pens, cages and containers shall:

- a) be constructed of durable, water-impermeable materials;
- b) be kept clean and cleaning frequencies be described, and all defective and redundant equipment be replaced or removed, as appropriate;
- c) be maintained in good repair and a detailed management programme for the replacement of defective and redundant equipment be documented;
- d) be escape-proof;
- e) protect the animals from climatic extremes;
- f) not cause injury to the animals;
- g) be large enough to ensure the animals' wellbeing; and
- h) be compatible with the behavioural needs of the species.

7.5.6.3 The population density of animals within cages, pens or containers and the placement of these in rooms shall be such that acceptable social and environmental conditions for the species can be maintained. Where it is necessary to individually house animals of a species which are normally kept in a social group, the conditions shall be managed so as to minimize the impact of social isolation. Animals shall be housed in these circumstances for the minimum time necessary. The individual housing of social species shall be subject to AEC approval.

7.5.6.4 Bedding and litter shall be provided if appropriate to the species or to the scientific study or teaching activity, and shall be comfortable, absorbent, safe, non-toxic, able to be sterilized if needed, and shall be suitable for the particular scientific or educational aims. Pregnant animals shall be provided with nesting materials where appropriate.

7.5.6.5 The AEC and relevant researchers or teachers shall be informed by the person-in-charge in advance of any planned changes to immediate environmental conditions since these might affect the welfare of the animals and the results of the scientific studies and teaching activities.

7.5.7 Environmental enrichment

NOTE In the past, sanitation, infection control and standardization have influenced cage design. Today, environmental enrichment has become the main consideration.

To list the many methods for environmental enrichment is beyond the scope of this standard, and the relevant considerable literature in this field should be consulted. Although there are universally applicable criteria, environmental enrichment should be approached in a species-specific fashion.

7.6 Species-specific minimum housing requirements

7.6.1 Amphibians (see annex B)

7.6.1.1 Dimensions of the tank and volume of water shall be large enough to allow frogs to:

- a) move and swim around;
- b) lie fully submerged, well away from the water surface;

NOTE About 1 L to 2 L of water is needed per frog at a depth of at least 6 cm to 12 cm to be able to fully cover the frogs.

- c) avoid excessive contact with other frogs;
- d) turn in any direction without impediment from tank walls or other frogs; and
- e) use any environmental enrichment items.

7.6.1.2 There shall be sufficient water surface area to allow breathing space for all the adult frogs to prevent drowning.

NOTE Adult frogs are lung breathers that often surface and sit on land. It would thus be advisable to provide a solid surface that protrudes above the water to encourage this behaviour.

7.6.1.3 Long narrow tanks are not acceptable since they restrict movements, limit social behaviour, and cause problems during feeding frenzies.

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7.6.2 Birds (see annex C)

7.6.2.1 General

7.6.2.1.1 Birds shall be housed in enclosures that facilitate and encourage a range of natural behaviour, including socialization, foraging and exercise.

7.6.2.1.2 All birds, especially those that spend a lot of time walking (quail and fowl), shall be housed on solid flooring, with suitable substrate.

7.6.2.2 Ducks and geese

7.6.2.2.1 Individual housing, unless justified for scientific or medical reasons, shall be avoided.

7.6.2.2.2 The minimum pen space for ducks and geese shall be sufficient to allow enough room for a full range of normal behaviour, including foraging, walking, running, and wing flapping (see table 2). The number of birds housed shall also be determined by age, size and weight.

Table 2 — Housing areas for waterfowl

1	2	3	4
Type of waterfowl	Area per bird		Minimum height m
	Group-housed m ²	Pair-housed m ²	
Ducks up to 1,2 kg	0,6	1	3
Ducks over 1,2 kg	0,9	1,5	3
Geese	1,0	1,5	3

7.6.2.3 Domestic fowl (*Gallus gallus domesticus*)

The minimum pen space for domestic fowl shall be sufficient to allow enough room for a full range of normal behaviour, including foraging, walking and running (see table 3).

Table 3 — Cage and feed trough sizes for domestic fowl

1	2	3	4	5
Body weight g	Area per bird		Minimum height cm	Minimum length of feed trough per bird cm
	Group-housed m ²	Pair-housed m ²		
Up to 300	0,15	0,5	30	3
301 to 600	0,2	0,5	40	7
601 to 1 200	0,3	1	50	15
1201 to 1 800	0,4	1	50	15
1 801 to 2 400	0,5	1,5	55	15
> 2 400	0,6	1,5	75	15

Note Adult male birds are considered in the same group as body mass >2 400.

7.6.2.4 Quail (*Coturnix coturnix*)

7.6.2.4.1 Quail shall be group-housed in either female or mixed sex groups (see table 4).

7.6.2.4.2 Cage height shall be from 20 cm to 30 cm.

Table 4 — Minimum floor space allocation for quail

1	2	3
Body weight g	Area per bird	
	Group-housed cm ²	Single-housed cm ²
< 75	<100	350
76 to 100	150	350
101 to 150	250	350
151 to 250	250	400

7.6.2.5 Pigeons (*Columbiformes*)

Strong justification on scientific grounds shall be provided if birds are to be housed singly in small cages.

7.6.2.6 Finches (*Fringillidae*)

7.6.2.6.1 Finches are extremely social and gregarious and shall never be housed singly unless there is compelling scientific justification or medical reasons.

7.6.2.6.2 The minimum cage space for finches shall be sufficient to allow enough room for a full range of normal behaviour, including foraging, walking and running (see table 5).

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Table 5 — Cage sizes for finches

1	2	3	4
Number of birds	Minimum pen area m ²	Minimum height m	Minimum number of feeders
Breeding pair	0,5	0,3	1
< 6	1	1	2
7 to 12	1,5	2	2
13 to 20	2	2	3
Each additional bird	0,05	2	1 per 6 birds

7.6.3 Cattle (see annex D)

7.6.3.1 The space allocation dimensions given in table 6 are for in-house holding facilities for cattle. Feedlot or pasture holding systems should follow the recommendations given for these systems such as in the *Recommended Codes of Practice and Factsheets for the Care and Handling of Farm Animals — Recommended Code of practice for Cattle*.

Table 6 — Stable or pen dimensions, stocking densities and minimum floor area requirements for cattle

1	2	3	4
Body weight kg	Minimum floor area per animal		Minimum length of feed rack or trough per head m
	Group-housed m ²	Single-housed m ²	
Up to 60 kg	1,5	2,2	0,30
61 to 100 kg	1,7	2,4	0,30
101 to 150 kg	1,9	2,8	0,35
151 to 200 kg	2,4	3,6	0,40
201 to 400 kg	3,8	5,7	0,55
Over 400 kg	5,3	8,0	0,65
Adult male	–	10,0	0,65

7.6.3.2 As a general rule, an animal shall have enough space to turn around in and to express normal postural adjustments, shall have ready access to food and water, and shall have enough clean-bedded and unobstructed area to rest in.

7.6.3.3 The AEC may agree to slightly smaller housing facilities if the animals have regular access to exercise or outdoor facilities during the day.

7.6.4 Cephalopods (see annex E)

A tank of 1 m diameter and 0,6 m depth shall be provided for five to ten cephalopods of weight 250 g to 1 000 g, and a tank of 2 m diameter and 0,6 m depth shall be provided for twenty to thirty cephalopods.

7.6.5 Dogs and cats (see annex F)

7.6.5.1 Sleeping areas

7.6.5.1.1 All kennels and cat pens should be provided with a raised sleeping area that is covered with a soft bedding material. Cats should be provided with one raised sleeping area per cat.

7.6.5.1.2 Facilities should consider providing beds and bedding for animals, both for comfort and for environmental enrichment.

7.6.5.2 Pen and cage sizes — Dogs (see tables 7 and 8)

7.6.5.2.1 Animal housing, whether for single or group housing, shall provide sufficient space for each animal to feed, sleep, sit, stand, lie with limbs extended, stretch and defecate.

7.6.5.2.2 Pre-weaned puppies and periparturient and suckling bitches shall not be housed on an open floor system. Dogs on open floor systems shall be provided with a raised comfortable solid surface for resting and sleeping.

7.6.5.2.3 Tables 7 and 8 show the recommended minimum sizes for the accommodation of dogs for scientific purposes:

Table 7 — Cage sizes for dogs

1	2	3	4
Body weight	Minimum floor area per dog		Minimum height
kg	Group-housed m ²	Single-housed m ²	m
< 20	2,0	4,0	2,0
> 20	4,0	8,0	2,0

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Table 8 — Pen, enclosure and cage sizes for various categories of dogs

1	2	3	4	5	6
Category	Length	Width	Height	Maximum number of animals	Area per animal
	m	m	m		m ²
Dog breeding pens	6,0	6	1,6 to 2,8	6	6,0
Dog exercise pens	12,5	6,15	1,6 to 2,8	8	9,6
Dog sick bay cage	1,9	1,3	3	1	2,47
Open dog pens under roof (15 kg to 20 kg)	3,5	1,6	3	1	5,6
Controlled environment dog cage (15 kg to 20 kg)	3	1,9	3	1	5,70
Dog exercise pen	19,5	4,1	3	5	15,99
Weaned puppies' pens (6 weeks to 3 months)	4,5	1,5	1,6	6	1,125
Weaned puppies' pens (3 months to 6 months)	4,5	1,5	1,6	2	3,375

7.6.5.3 Pen and cage size — Cats (see tables 9, 10 and 11)

7.6.5.3.1 Communal housing

Cats shall have access to an exercise area, if not adequately provided for in the housing pen.

Table 9 — Controlled environment cat room size

1	2	3	4	5
Length	Width	Height	Maximum number of adult animals	Area per animal
m	m	m		m ²
4,5	3,5	3	12	1,31

Table 10 — Cat exercise run size

1	2	3	4	5
Length	Width	Height	Maximum number of adult animals	Area per animal
m	m	m		m ²
4,9	3,6	3	15	1,18

7.6.5.3.2 Individual housing

7.6.5.3.2.1 Cats shall have access to an exercise run of minimum size 1,8 m × 1,2 m × 600 mm.

7.6.5.3.2.2 Table 11 shows the recommended minimum sizes for individual housing units.

Table 11 — Cat pen floor space per animal

1	2
Body weight kg	Minimum floor space per animal m ²
< 1	0,5
< 2	0,55
< 3	0,65
> 3	0,75

7.6.5.3.2.3 Shelves shall be a minimum of 0,5 m off the ground, with enough space for a cat to comfortably stand either on or below the shelf without interfering with its natural behaviour. The shelf shall be large enough for a cat to lie down on it without its limbs protruding over the edge.

7.6.5.3.2.4 An individual housing unit shall also contain a night box of minimum size 600 mm × 600 mm × 600 mm.

7.6.5.3.3 Queen and litter housing

7.6.5.3.3.1 For a queen and litter up to three weeks of age, the minimum space requirement is 1 m² of usable floor space, with a minimum height of 100 cm. A separate shelf area shall be provided to allow the queen some personal space distant from the litter.

7.6.5.3.3.2 For a holding queen and litter from three weeks of age to weaning, the minimum pen size shall be an area of at least 2 m² that is at least 2 m high.

7.6.6 Fish (see annex G)

Aquatic environments shall be so designed as to meet the established physical and behavioural requirements of the species of fish, and their life stages, in terms of social grouping and housing criteria.

7.6.7 Horses (see annex H)

7.6.7.1 The space allocation dimensions given in table 12 are for in-house holding facilities for horses.

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Table 12 — Stable or pen dimensions, stocking densities and minimum housing requirements for horses

1	2	3
Type of housing	Weight of animal kg	Minimum floor area per animal m ²
Single box	–	10
Group box	< 200	6,5/horse
Group box	> 200	7,5/horse
Foaling box	–	15

7.6.7.2 As a general rule, an animal shall have enough space to turn around in and to express normal postural adjustments, shall have ready access to food and water, and shall have enough clean-bedded and unobstructed area to rest in.

7.6.7.3 The AEC may agree to slightly smaller housing facilities if the animals have regular access to exercise or outdoor facilities during the day.

7.6.8 Non-human primates (baboons and vervet monkeys) (see annex I)

7.6.8.1 General

7.6.8.1.1 The space allocation dimensions given in table 13 are for in-house holding facilities for non-human primates.

7.6.8.1.2 Minimum cage sizes shall be based on adult male dimensions (see table 13)

7.6.8.1.3 The design of the cage partition shall make allowance for grooming with compatible neighbours (for example, via a mesh communication panel within the solid partition).

7.6.8.1.4 The design of the cage shall enable foraging (for example, by placing a foraging tray underneath the cage).

7.6.8.1.5 Creative and innovative deviations from the recommended cage size shall be documented and fully justified in the written proposal to the AEC (see 5.2.4), which shall be made available for inspection.

7.6.8.2 Vervet monkeys

Cages for vervet monkeys (see table 13) shall be fitted with a resting perch of at least 30 cm in depth placed 60 cm above the cage floor along the entire width of the cage so that an adult male will be able to sit comfortably on and under the perch.

7.6.8.3 Baboons

Cages for baboons (see table 13) shall be fitted with a resting perch of at least 50 cm in depth placed 90 cm above the cage floor along the entire width of the cage. If the cage is 2 m in height, the perch shall be 130 cm above the cage floor.

Table 13 — Cage or pen dimensions, stocking densities and minimum housing requirements for non-human primates

1	2	3
Vervet monkeys	Minimum floor area m ²	Minimum cage height cm
Adult	0,6	100
Pair	1,2	100
Communal housing (per animal)	1,0 to 1,2	200
Baboons		
< 25 kg	1,6	150
> 25 kg	1,6	200
Pair	3,2	200
Communal housing (per animal)	2,0 to 2,5	200
NOTE 1 Communal housing for vervet monkeys – The formula of 0,63 m ² (derived from a cage size of 0,9 m × 0,7 m) cannot be applied to full communal housing because of increased social complexity and density. Therefore, 1,0 m ² to 1,2 m ² per animal is more appropriate in this situation, depending on the size of the individuals. The height of communal cages should be a minimum of 2 m.		
NOTE 2 Communal housing for baboons – The same formula as for vervet monkeys, but consideration should be given to the fact that baboons are terrestrial and need less height but more space, and to the different social structure and group dynamics of baboons.		

7.6.9 Pigs (see annex J)

7.6.9.1 As a general rule, an animal shall have enough space to turn around in and to express normal postural adjustments, shall have ready access to food and water, and shall have enough clean-bedded and unobstructed area to rest in (see table 14).

Table 14 — Cage or pen dimensions, stocking densities in group housing and minimum housing requirements for pigs

1	2	3	4
Body weight kg	Minimum pen size m ²	Area per animal m ²	Minimum length of feed rack or trough per head m
< 10	–	–	–
11 to 30	1,2	0,3	0,2
31 to 50	1,6	0,4	0,25
51 to 75	1,8	0,9	0,30
76 to 100	3,0	1,2	0,35
> 100	4,5	2,0	0,40

NOTE The use of partial or fully slatted housing systems is not recommended for laboratory animal facilities unless approved by the AEC.

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7.6.9.2 The AEC may agree to slightly smaller housing facilities if the animals have regular access to exercise or outdoor facilities during the day.

7.6.10 Rabbits and guinea pigs (see annex K)

7.6.10.1 The minimum pen space for rabbits and guinea pigs shall be of enough room to allow a full range of normal behaviour (see tables 15 and 16).

7.6.10.2 Where mesh floors are intended, a suitable mesh shall be used to minimize the risk of injury to the animal's feet and legs. The flooring material shall be blunt and thick enough to prevent foot ulcerations (pododermatitis). Perforated bottom cages are preferred over wire-bottom cages. With the latter, a part of the cage shall be provided with solid floor cover to allow animals a solid resting surface.

7.6.10.3 If solid sided cages are used, these shall be positioned such that the animals have visual contact with other rabbits. It is preferable that at least one side of the cage be mesh or transparent to give improved visual contact, and thus reduce disturbance to the animals.

Table 15 — Cage or pen dimensions, stocking densities and minimum housing requirements for breeding rabbits and guinea pigs (including litters)

1	2	3
Breeding category	Minimum floor area cm ²	Minimum cage height cm
Rabbits		
Doe and litter < 3 kg	4 000	45
Doe and litter > 3 kg	6 400	45
Guinea pigs		
Mother and litter Monogamous pair and litter	1 500	23
Female in harem	1 000	23

Table 16 — Cage dimensions, stocking densities and minimum housing requirements for non-breeding rabbits and guinea pigs

1	2	3	4	5
Rabbits				
Body weight kg	Group-housed		Single-housed	
	Minimum floor space cm ²	Minimum cage height cm	Minimum floor space cm ²	Minimum cage height cm
< 2,0	1 500	40	2 000	40
2,0 to 2,5	2 000	45	3 000	45
2,6 to 3,0	2 500	45	3 000	45
3,1 to 3,5	3 000	45	4 000	45
3,6 to 4,0	4 000	45	4 000	45
4,1 to 6,0	5 400	45	5 400	45
> 6,0	6 000	45	6 000	45
Guinea pigs				
Body weight g	Group-housed		Single-housed	
	Minimum floor space cm ²	Minimum cage height cm	Minimum floor space cm ²	Minimum cage height cm
< 150	200	20	700	20
151 to 250	300	20	700	20
251 to 350	400	20	700	20
351 to 450	500	20	700	20
451 to 550	600	23	700	23
> 550	700	23	800	23

7.6.11 Rodents (Mice, rats and hamsters) (see annex L)

7.6.11.1 The minimum pen space for rodents shall be of enough room to allow a full range of normal behaviour (see tables 17 and 18).

7.6.11.2 Where mesh floors are intended, a suitable mesh shall be used to minimize the risk of injury to the animal's feet and legs. The flooring material shall be blunt and thick enough to prevent foot ulcerations (pododermatitis). Perforated bottom cages are preferred over wire-bottom cages. With the latter, a part of the cage shall be provided with solid floor cover to allow animals a solid resting surface.

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Table 17 — Cage dimensions, stocking densities and minimum housing requirements for breeding rodents (including litters)

1	2	3
Breeding category	Minimum floor area cm ²	Minimum cage height cm
Mice		
Monogamous pair (outbred/inbred)	300	12
Trio (inbred)	300	12
For each additional female plus litter	an additional 180	12
Rats		
Mother and litter Monogamous pair and litter	900	18
Hamsters		
Mother and litter Monogamous pair and litter	650	15

Table 18 — Cage dimensions, stocking densities and minimum housing requirements for non-breeding rodents

1	2	3	4	5
Body weight g	Group-housed		Single-housed	
	Minimum floor space cm ²	Minimum cage height cm	Minimum floor space cm ²	Minimum cage height cm
Mice				
< 20	30	12	200	12
21 to 25	45	12	200	12
26 to 30	60	12	200	12
> 30	100	12	200	12
Rats				
< 100	75	18	500	18
101 to 150	100	18	500	18
151 to 250	150	18	500	18
251 to 350	250	20	700	20
351 to 450	300	20	700	20
451 to 550	350	20	700	20
> 550	400	20	800	20
Hamsters				
<60	80	15	300	15
61 to 90	100	15	300	15
91 to 120	120	15	300	15
>120	165	15	300	15

7.6.12 Sheep and goats (see annex M)

7.6.12.1 The space allocation dimensions given in table 19 are for in-house holding facilities for sheep and goats. Feedlot or pasture-holding systems should follow the recommendations given for these systems as in the *Recommended Code of Practice for the Care and Handling of Farm Animals: Sheep*.

Table 19 — Pen dimensions, stocking densities and minimum floor area requirements for sheep and goats

1	2	3
Type of animal	Minimum floor area per animal	
	Group-housed m ²	Single-housed m ²
Sheep		
Dry ewes	0,9	1,8
Pregnant ewes	1,4	1,8
Ewe and lamb(s)	1,5	2,0
Rams	1,0	2,0
Goats^a		
Does (nanny goats)	1,7	2,0
Young kids	0,5	1,2
Weaned kids	0,9	1,2
Bucks (billy goats)	3,7	4,5
^a The requirements presented here are for meat and dairy goat breeds. Dwarf goats can be housed at lesser floor area requirements, and special adjustments shall be made for fibre goats.		

7.6.12.2 As a general rule, an animal, at a minimum, shall have enough space to turn around in and to express normal postural adjustments, shall have ready access to food and water, and shall have enough clean-bedded and unobstructed area to rest in.

7.6.12.3 The AEC may agree to slightly smaller housing facilities if the animals have regular access to exercise or outdoor facilities during the day.

7.6.13 Terrestrial reptiles (see annex N)

Housing facilities shall be suitable for the size and the physiological needs of the reptile.

7.7 Management and staff

7.7.1 Person-in-charge of breeding and holding facilities

7.7.1.1 Animal acquisition, breeding and holding facilities shall be supervised by persons with appropriate veterinary or animal care qualifications and experience.

7.7.1.2 The person-in-charge shall be responsible for the management of the day-to-day care of the animals in holding and breeding facilities and for supervising the work of other staff in the facility, and shall act as a liaison between the researcher and the facility staff.

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7.7.1.3 The person-in-charge shall ensure that there is reliable monitoring of the wellbeing of all animals by other staff, and shall be knowledgeable regarding animal husbandry and signs of pain, distress and illness specific to each species housed. After animals are allocated to an approved study, the researcher shall have joint responsibility for ensuring adequate monitoring of the animals' wellbeing.

7.7.1.4 The person-in-charge shall ensure that ill or injured animals that are not allocated to approved studies are treated promptly and that the cause of death is investigated for all animals that die unexpectedly.

7.7.1.5 The person-in-charge shall contribute to the development and maintenance of the institution's animal care policies and procedures, and the quality assurance programme and documentation.

7.7.1.6 The person-in-charge shall ensure that staff are provided with appropriate protective clothing and equipment, maintain high standards of personal hygiene, do not eat, drink or smoke in animal areas, and have all required routine vaccinations, particularly against tetanus and other zoonoses. A staff health monitoring and screening programme, including allergy-testing and routine required vaccinations, shall be in place.

7.7.1.7 The person-in-charge shall document procedures used in the management of breeding and holding facilities. These procedures shall take into account:

- a) the requirements of the species held;
- b) the scientific studies and teaching activities being conducted;
- c) the health and safety of the staff;
- d) transport, quarantine, isolation and disposal of animals;
- e) routine husbandry;
- f) prevention, diagnosis and treatment of disease;
- g) monitoring of health status;
- h) genetic constitution; and
- i) physical environmental factors.

These procedures shall be made known to all staff involved in the care and use of the animals and shall be reviewed regularly.

7.7.1.8 The person-in-charge shall be responsible for developing and implementing programmes for assessing and addressing the psychological wellbeing of the animals. Environmental enrichment programmes shall be applied in accordance with recognized current standards for each species, and shall be compulsory for all primates (see annexes B to N).

7.7.1.9 The person-in-charge shall ensure that adequate records are maintained of:

- a) the source, care, allocation, movement between locations, use and disposal of all animals, and any diseases developed;
- b) the fertility, fecundity, morbidity and mortality in breeding colonies, in order to monitor the management of the colonies and to assist detection of the origin and spread of disease; and

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c) the health status, genetic constitution and the physical environment of the animals, when information on these is required.

7.7.1.10 Records maintained by the person-in-charge shall be made available to researchers and teachers, the AEC, and the institutional and regulatory authorities.

7.7.1.11 The person-in-charge shall ensure that researchers and teachers, the AEC and the institutional and regulatory authorities are informed of any changes to the conditions under which animals are held and which might affect the results of their studies.

7.7.2 Staff

7.7.2.1 High standards of animal care shall be maintained by the appointment of a sufficient number of well-trained staff. Staff working with animals in a holding facility shall be appropriately instructed in the latest methods for care and maintenance of those animals, how they might affect the animals' wellbeing, and how their actions might affect the outcome of scientific studies and teaching activities.

7.7.2.2 Although institutions should only employ staff appropriately qualified in laboratory animal science to perform the tasks of the laboratory animal technician, some husbandry practices might be performed by less qualified staff such as cleaners or caretakers (or both). Institutions shall thus encourage and promote the formal training of such personnel in laboratory animal science and technology. The institution shall ensure that a training programme is in place and that the content of the programme is suitable and relevant and that the person conducting the training has verifiable credentials.

7.7.2.3 Staff employed in the care of animals shall be instructed in how to recognize at an early stage changes in animal behaviour, performance and appearance.

7.7.2.4 New appointees who will care for animals shall be appropriately instructed in their duties and in institutional policy.

7.7.2.5 Staff shall be informed of the important zoonotic diseases of animals under their care and of precautions that shall be taken. A health monitoring and screening programme for all staff that handle animals is recommended in the interest of both staff and animals. This programme shall include allergy and tuberculosis (TB) testing, and appropriate vaccinations against tetanus, hepatitis A and B, and rabies.

7.7.2.6 If employed, a full-time veterinarian shall have a described task analysis. A part-time veterinarian shall provide a schedule of visit frequencies.

7.7.2.7 All institutions, AECs and laboratory animal facilities shall have an Occupational Health and Safety (OHS) manual, and an appointed OHS officer to monitor the workplace safety. Routine OHS checks shall be made at a prescribed frequency and all records filed for audit purposes. These records shall contain all recommendations for improvements, incident reports and corrective actions.

7.7.2.8 Staff shall be allocated to a duty roster that covers animal care duties for weekends, public holidays and after-hour periods.

7.7.3 Routine husbandry procedures

7.7.3.1 Husbandry procedures (for example, clipping coats and nails, and vaccinations) that are not part of an approved study shall be performed by competent personnel.

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7.7.3.2 Routine husbandry procedures on farm animals shall be carried out in accordance with relevant standards of practice and the Animal Protection Act, 1962 (Act No. 71 of 1962).

7.7.3.3 Routine husbandry procedures shall be documented and be made available to the AEC.

7.7.4 Identification of animals

7.7.4.1 Animals shall be identified by a method such as a tattoo, a neckband, an individual tag, an electronic numbering device, a physical mark, or by a label or marking attached to the cage, container, pen, yard or paddock in which the animals are kept.

7.7.4.2 The person-in-charge of the facility shall be responsible for ensuring that animals are identifiable (see 7.7.4.1) before being allocated to an approved study. Thereafter, the identification of animals within a study will be the responsibility of both the person-in-charge and the researcher.

NOTE The method of identification should be reliable and cause the least stress possible.

7.7.5 Disposal of animal carcasses and waste

Appropriate provision shall be made for prompt and sanitary disposal of animal carcasses and waste material in accordance with the Occupational Health and Safety Act, 1993 (Act No. 85 of 1993), local council by-laws and community standards. Methods of disposal and storage on site shall be approved by the institutional biosafety officer, or the IBSC (or both), and shall be monitored by the person-in-charge of the facility. SOPs shall clearly describe these activities.

8 Wildlife studies

8.1 General

This clause refers to free-living animals or those captured from free-living populations, including both indigenous and non-indigenous (exotic) species, and feral species. All scientific studies and teaching activities that involve wildlife, which are performed in order to acquire, develop or demonstrate knowledge or techniques in any scientific discipline, require AEC approval.

8.2 Wildlife captured from natural habitats

8.2.1 Many species of wildlife are protected by provincial ordinances. Conservation authorities shall be consulted when these species are required. Permits are usually necessary to collect, keep, release or kill protected fauna, and further permits are usually required to import or export such species between or through provinces. Any conditions imposed on permits shall be observed.

8.2.2 Observation studies on free-living animals have the potential to cause adverse effects because of interference with the normal behaviour of the animals, particularly if there is an effect on the rearing of the young. If interference with animals is substantial, the continuation of the procedure shall be reviewed.

8.2.3 Animals shall only be taken from natural habitats if animals bred in captivity are not available or are unsuitable for the specific scientific purpose.

8.2.4 Researchers and teachers shall recognize that field studies have the potential to cause disturbance to the habitat and adversely affect the resources available to both target and non-target species. Efforts shall be made to consider the potential for such disturbance and minimize it, both before and during the study.

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8.2.5 Repetition of studies shall be avoided. However, because of the complexity of the interactions between wildlife populations and the environment, replication and long-term studies might be necessary and essential for a thorough understanding of the species or the ecosystem and their management.

8.2.6 Reuse of individual animals requires AEC approval (see 6.3.1.3.1). However, the nature of wildlife field studies is such that individual animals might be recaptured, and AECs shall be made aware of this possibility by the researcher or the teacher.

8.2.7 The capture, holding, transport, or handling and release of animals shall be in accordance with the principles outlined in this standard.

8.2.8 Researchers and teachers shall be aware that the effects of a series of stressors, such as trapping, handling, transport, sedation, anaesthesia, marking and sampling, can be cumulative, and might produce severe, possibly fatal, consequences. An assessment of the potential sources of stress and management plans to eliminate or minimize distress shall form part of the proposal submitted to the AEC.

8.2.9 The risk of disease transmission shall be taken into account and appropriate steps taken to clean all materials and equipment used in the capture, holding, transport and manipulation of animals.

8.2.10 A method of evaluating the quality and health status of captured animals shall be described.

8.3 Capturing of wildlife

8.3.1 General

Capture is stressful to animals, and steps shall be taken to minimize the disruption of the population and any distress caused by the capturing process. There shall be a careful choice made regarding suitable capture techniques, skilled persons shall be used, and appropriate and safe enclosures or caging shall be provided after capture. Animals shall be monitored for signs of distress following capture at a frequency and level which will protect the welfare of the animals and remedial steps taken if necessary.

8.3.2 Use of traps

8.3.2.1 If trapping is to be used, the study proposal shall include details as to how the traps will be managed to minimize the impact on both target and non-target species, taking into account issues such as:

- a) the time the animals will spend in these traps;
- b) the protection of animals from predators or from being parasitized;
- c) the protection from environmental effects such as dehydration, extremes of environmental temperature and drowning;
- d) the deprivation of food and water;
- e) the potential for impact via disruption of social structure;
- f) the potential for impact on their dependent young;

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- g) the construction of the traps (i.e. conformation of the walls and lids, covers or grids);
- h) the appropriate size of the traps (i.e. diameter and depth); and
- i) the deactivation of the traps when not in use or no longer required.

8.3.2.2 Any form of trapping has the potential to cause harm or distress to animals if the trap is not managed correctly. The trapping technique shall be appropriate to the species, shall be justified in the study proposal, and shall be approved by the AEC. For the correct means of capture for each species, *The Capture and Care Manual* and SANS 10331 shall be consulted.

8.3.2.3 When traps or nets are used to capture animals in water, they shall be arranged in such a way as to avoid drowning.

NOTE This refers to traps used to catch aquatic animals (also called fish-traps, hoop-nets or bow-nets). Drowning refers to aquatic species such as the African-clawed frog (*Xenopus laevis*) that has to surface for a breath of fresh air; if the trap is submerged this will make surfacing impossible and the frog will drown.

8.3.2.4 The use of wet pitfall traps is unacceptable for the capture of vertebrates. When wet pitfall traps are used for the capture of invertebrates, these traps shall be managed so as to minimize the inadvertent capture of vertebrates.

8.3.2.5 The selling, inspection and laying of traps and other devices for the purposes of capturing and destroying animals is subject to provisions under the Animals Protection Act, 1962 (Act No. 71 of 1962) and different Nature Conservation ordinances.

8.3.3 Non-trap capture

A wide variety of non-trap capture techniques can also be used in field studies. Similar principles apply as those detailed in 8.3.2 for traps. The skill of the operator is essential to ensure minimal impact on target and non-target species. For the correct means of capture for each species, *The Capture and Care Manual* and SANS 10331 shall be consulted.

8.3.4 Handling and restraint of wildlife

8.3.4.1 Captured free-living animals shall be handled in a way which minimizes distress and the risk of injury or stress-induced disease.

8.3.4.2 In particular, the risk of injury or stress-induced disease shall be minimized by:

- a) firm and quiet handling;
- b) limiting handling and restraint time to the minimum needed to achieve the scientific or educational objectives;
- c) using sufficient competent persons to restrain animals and prevent injury to either animals or handlers;
- d) using techniques and timing appropriate to the species; and
- e) using, if appropriate, chemical restraint (including sedation or tranquillization) if animals are to be held for more than a short time.

8.3.4.3 Wherever possible, the long-term and short-term consequences of capture, handling and restraint shall be recorded.

8.3.5 Holding and release

8.3.5.1 The time for which an animal is held shall be minimal, and shall be consistent with the aims of the scientific study or teaching activity.

8.3.5.2 Animals shall be held in a way that minimizes stress or injury (or both). Knowledge of available information on the normal behaviour of the species and likely response to captivity is essential and shall form the basis for management practices.

8.3.5.3 Holding areas and containers shall be safe, quiet and hygienic.

8.3.5.4 Closed confinement devices, including bags and crates, shall:

- a) allow animals to rest comfortably;
- b) minimize the risk of escape or injury;
- c) be adequately ventilated;
- d) maintain animals within appropriate levels of ambient temperature and humidity; and
- e) minimize the risk of disease transmission.

8.3.5.5 Release shall be at the site of capture, unless an alternative site is justified in the study proposal.

8.3.5.6 Time of release shall be consistent with the species' usual time of movement. Individuals shall be released safely, particularly if the time of day for release is less than optimal.

8.3.5.7 At the time of release, all reasonable steps shall be taken to protect animals from injury and predation.

8.3.6 Transport

8.3.6.1 Wildlife are particularly susceptible to transport stress and all reasonable steps shall be taken to minimize that stress. The general principles for transport detailed in 7.1, 7.2 and 7.3 apply, and particular reference shall be made to the wildlife section of the IATA regulations, to *The Capture and Care Manual*, and to SANS 10331.

8.3.6.2 Stress during transport can be minimized by:

- a) ensuring the appropriate size, design and construction of transport containers;
- b) limiting animals from exposure to temperature extremes, noise, visual disturbance and vibration;
- c) providing, if appropriate for the species, an inner shelter within the transport container;
- d) ensuring that animals are separated where there is incompatibility of species, age, size, sex or reproductive status;
- e) preventing unnecessary handling; and
- f) administering sedatives and tranquillizers, if appropriate, by suitably trained persons.

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8.3.7 Identification

8.3.7.1 The method for identification of individual animals shall be that which causes the least physiological and psychological distress within the context of the research proposal and the least interference with the normal behaviour of the animal.

8.3.7.2 Identification methods shall not compromise an animal's survival capabilities should it be released.

8.3.7.3 Identification methods shall be approved by the AEC.

8.3.8 Field techniques

A wide range of minor procedures are used in the field which involve only capture and release, possibly facilitated by tranquillizers or short-acting anaesthetics. Such procedures could include identification (for example, leg banding, ear tagging, microchipping and radio-tracking devices), examination, measurement, and sampling (for example, hair, feathers, scales, blood and stomach contents of birds). The procedures may be carried out, subject to AEC approval, but only once the following criteria are considered:

- a) all procedures shall be conducted by appropriately qualified and experienced persons, using clean equipment in each instance, in an uncontaminated area;
- b) equipment necessary to provide for the health and welfare of the animals and relief of pain shall be readily available;
- c) recovery to full consciousness shall occur in an area in which animals can be readily observed, can maintain normal body temperature, and are protected from injury or predation;
- d) the potential impact of the procedures on dependent young shall be minimized; and
- e) the methods and equipment used shall be appropriate to the species.

8.3.9 Voucher specimens

To be optimally useful, a voucher specimen shall become part of a publicly accessible reference collection. Therefore:

- a) voucher specimens should be lodged with a museum or other institution that can properly house and curate them, and make them available for further studies;
- b) consultation with the institution shall take place before collection to ensure that there is an understanding of the proper preservation and holding techniques, the necessary equipment and the essential data required; and
- c) proper documentation of the specimens, including reasons for collection, is essential and data shall be maintained together with the specimens.

8.3.10 Wildlife interaction studies

8.3.10.1 Wildlife interaction studies might involve work in the field or work under laboratory conditions, and can include interaction between species (for example, predator/prey), within species (for example competition) or between species and habitat.

8.3.10.2 The primary ethical considerations with wildlife interaction studies are the degree of manipulation required to set up the interaction, and the additional effect of the observer(s) on the interaction.

8.3.10.3 Wherever possible, efforts shall be made to reduce the number of animals used for the study, and alternatives should be considered.

8.3.10.4 Field studies shall include the monitoring of animals outside the study, including other species that might be influenced by the manipulation.

8.3.10.5 In studies of predatory encounters, unstaged natural encounters in the field shall be used wherever possible. If staging is required then alternative models of predators or products of predators (for example, body odour and faeces) rather than live animals shall be used wherever possible.

8.3.11 Feral animal studies

8.3.11.1 This standard applies equally to feral animals.

8.3.11.2 The primary purpose of studies that involve feral animals is often to measure the efficacy of methods of killing or control. AECs need to be aware of this and weigh up the study justification carefully. Such justification shall address appropriate animal welfare concerns (see also 5.2.4.3(m)).

8.3.11.3 Relevant, specific literature, *The Capture and Care Manual*, standards such as SANS 10331, and the Animals Protection Act, 1962 (Act No. 71 of 1962) shall be consulted relating to the capture, holding and transport of wildlife.

8.3.11.4 Where wildlife captured from natural habitats are required to be humanely put to death, this shall be done in accordance with the welfare provisions of this standard and recognized euthanasia methods.

9 Care and use of farm animals for scientific studies and teaching activities

NOTE Teaching includes demonstrations.

9.1 General

This clause refers to the special considerations involved when farm animals are used to acquire, develop or demonstrate scientific knowledge and techniques. The intention is to clarify when AEC approval is required for the use of farm animals.

9.2 General requirements

9.2.1 Unless specifically exempted by an AEC, the care of farm animals managed by institutions shall at least comply with the *Standards of Practice for the Welfare of Farm Animals* published by the Livestock Welfare Co-ordinating Committee.

9.2.2 AEC approval is required when farm animals are used to acquire, develop or demonstrate knowledge and techniques, including their use for the production of biological products. The only exceptions to this are defined in 9.2.3.

NOTE 1 This includes standard husbandry procedures or normal farming practices such as mulesing, tail docking and beak trimming when these studies are being researched or taught.

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NOTE 2 Biological products do not include food or fibre.

NOTE 3 AEC approval is also not required when inspectorial staff are undertaking routine regulatory studies such as lice examinations, disease surveillance, tick control and sale yard work.

9.2.3 If all of the following apply then AEC approval is not required for agricultural extension or veterinary work that involves routine procedures:

- a) the animals are on their home property;
- b) the procedures occur normally as part of routine management;
- c) the animals are not subjected to anything additional to that which would occur in routine management; and
- d) the teacher or demonstrator is competent and registered or authorized (or both) by the relevant statutory body (i.e. the South African Veterinary Council (SAVC), the Health Professions Council of South Africa (HPCSA) and the South African Council for Natural Science Professions (SACNASP)) to carry out the procedure.

9.2.4 An annual return detailing the number of all animals maintained at the institution and the purposes for which they are kept (including grass eaters and breeding stock) shall be provided to the AEC (see 5.2.9.2).

9.3 AEC applications

9.3.1 AEC approval is required for teaching or demonstrating routine procedures not covered in 9.2.3.

9.3.2 To simplify AEC applications, institutions might require the development and use of SOPs. Once approved by the AEC, the SOPs may be referred to in a study proposal as a means of providing required information on techniques.

NOTE In developing an SOP, the principles of reduction of animal numbers, the refinement of procedures to reduce the impact on the animal and the replacement of animals by alternative, non-animal techniques should be included, where possible.

9.3.3 SOPs may be categorized on the basis of their likely impact on the animal. The researcher or teacher shall possess the appropriate skills and experience to carry out the procedures outlined in the study proposal.

NOTE Animal use severity categories consider the likely welfare impact on animals or the skill required to undertake the procedure (or both). The concept is explained in *Laboratory Animal Science*, January 1987 (Special issue), p 12.

9.3.4 All proposals for scientific studies or teaching activities not exempt under 9.2.3 or covered in 9.3.2 require a full AEC application as outlined under written proposals (see 5.2.4).

9.4 Teaching and demonstration requirements for all farm animals

NOTE This includes commercial ventures held on private property for teaching livestock techniques.

9.4.1 Facilities shall be available to treat animals that might be injured. Treatment might range from a minor procedure to euthanasia.

9.4.2 If animals are to be handled then there shall be a competent person present to protect animals from injury or distress. Animals that do not adapt to the situation shall be removed.

10 The use of animals to demonstrate knowledge or techniques in scientific disciplines in schools and tertiary institutions

10.1 General

This clause refers to the special ethical considerations and issues of responsibilities that shall be addressed when animals are used to demonstrate knowledge or techniques in any scientific discipline in schools and tertiary institutions. The purpose is to emphasise the principles most relevant to schools and tertiary institutions.

10.2 General principles

10.2.1 Animals shall be used for teaching activities only when there are no suitable alternatives for achieving the educational objectives.

10.2.2 All teaching activities which involve the use of animals shall have approval by an AEC which is satisfied that there is no suitable alternative to the use of animals, and that the number of animals involved and the impact on them is minimized.

10.2.3 Students shall be given the opportunity to discuss the ethical, social and scientific issues that are involved in the use of animals for scientific studies and teaching activities. Where students are involved in the use of animals as part of their professional training, curricula in the academic discipline involved shall include material on such issues.

10.3 Responsibilities of lecturers and teachers

10.3.1 The teacher or person-in-charge of the students shall be responsible for the care and use of the animals from their time of acquisition to the time of disposal and shall:

- a) ensure that all care and use of the animals is in accordance with the provisions of this standard and all relevant legislation, standards and guidelines (see clause 1);
- b) have relevant training and qualifications;
- c) identify whether methods which might replace, reduce or refine the use of animals and which are compatible with the educational objectives are available and, if so, incorporate such methods into the proposed studies;
- d) obtain AEC approval before the scientific studies and teaching activities commence and shall ensure that scientific studies and teaching activities are conducted as directed and approved by the AEC; and
- e) ensure that there is close, competent supervision of all students.

10.3.2 The lecturer or teacher responsible shall ensure that, when they are directly involved, students are instructed in the appropriate methods of handling and caring for animals and shall demonstrate their ability to perform the necessary tasks with care and competence.

10.3.3 Persons supervising students who are undertaking training in research shall ensure that before using animals, the students receive appropriate instruction in the ethical and legal responsibilities involved in the use of animals for scientific purposes, as well as in the appropriate

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methods for animal care and use. The person supervising such students shall be responsible for the welfare of the animals used by those students.

10.4 Animals used in primary and secondary schools

10.4.1 All primary and secondary schools shall have access to an AEC. This might include the establishment of regional, provincial or central AECs for schools.

10.4.2 The head of the school shall ultimately be responsible for ensuring compliance with the requirements in 10.4.

10.4.3 The following teaching activities shall not be carried out in primary or secondary schools:

- a) surgical procedures;
- b) induction of infectious diseases;
- c) production of nutritional deficiency giving rise to distress;
- d) exposure to stimuli which cause distress; and
- e) administration of toxins, ionising radiation or other biohazardous materials.

10.4.4 When the purpose of the activity is for students to interact with animals, the observation of animals in purpose-built facilities, in their natural environment or under field conditions shall be considered as an alternative to the temporary introduction of animals to the school.

10.4.5 Mechanisms shall be put in place to ensure that all use of animals in schools is in compliance with the principles of this standard. This might include:

- a) the establishment of a policy committee;
- b) the designation of a person at each school who will be responsible for promoting awareness of these principles;
- c) the acquisition or development of detailed guidelines; and
- d) appropriate teacher training.

10.4.6 Detailed guidelines and complete animal care records shall be available in schools for inspection at all times.

10.4.7 Students shall not be allowed to take animals home unless there is clear written undertaking from parents that the animals will be cared for adequately and responsibly.

10.4.8 Animals shall not be held for longer than necessary. If it is impossible to return the animals to their natural habit or to re-home them, such animals shall be humanely put to death by a qualified veterinarian. Arrangements for regular and on-going monitoring shall be made and holding facilities shall be secure against human or animal interference. Since school premises are largely unoccupied for part of each day, on weekends and on vacations, feeding and care of animals and security requires special attention during these periods.

Annex A

(informative)

Genetic and microbiological status of laboratory animals

A.1 The genetic status of laboratory animals

A.1.1 General

A large number of inbred, congenic and mutant strains as well as outbred stocks are in existence. In order to avoid confusion amongst breeders and suppliers of such animals, certain rules and guidelines have been set describing the genetic status (gene nomenclature, different types of inbred strains and outbred stocks) of these animals.

A.1.2 Genetic status definitions

A.1.2.1 Inbred strains

A strain shall be regarded as inbred when it has been mated brother x sister (hereafter called b x s) for 20 or more consecutive generations (F₂₀), and can be traced to a single ancestral breeding pair in the 20th or subsequent generations. Parent x offspring matings may be substituted for b x s matings provided that, in the case of consecutive parent x offspring matings, the mating in each case is to the younger of the two parents.

Inbred animals are homozygous and isogenic. From a study viewpoint, inbred animals are uniform, and there are no genetic variables between individuals of the same inbred strain (i.e. one less variable is added to the study design).

A.1.2.2 Substrains

Inbred strains may be subdivided into substrains. An established inbred strain is considered to be divided into substrains when known or probable genetic differences become established in separate branches. Such differences could arise by residual heterozygosity at the time of branching, mutation, or contamination. Hence, substrains should be considered to be formed:

- a) when branches are separated before F₄₀ (i.e. after 20 to 40 generations of b x s matings). In such cases, residual heterozygosity may be present;
- b) when a branch is known to have been maintained separately from other branches for 100 or more generations from their common ancestor. The existence of differences arising by mutation is then highly probable; and
- c) when genetic differences from other branches are discovered. Such differences could arise by any of the three means given (i.e. branching, mutation or contamination). Contamination is likely to lead to numerous genetic differences and can thus be distinguishable from mutation. If contamination is thought likely, the strain should be renamed (for example, A2G; a strain arising by contamination of strain A when maintained by Glaxo (G)).

A.1.2.3 Recombinant inbred (RI) and recombinant congenic (RC) strains

A.1.2.3.1 Recombinant inbred strains

RI strains are formed by crossing two inbred strains, followed by 20 or more generations of b x s mating.

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A.1.2.3.2 Recombinant congenic strains

RC strains are formed by crossing two inbred strains, followed by a few (usually two) backcrosses to one of the parental strains (the "background" strain), with subsequent inbreeding without selection for specific markers.

NOTE RC strains should be regarded as fully inbred when the theoretical coefficient of inbreeding approximates that of a standard inbred strain. For this purpose, one generation of backcrossing will be regarded as being equivalent to two generations of b x s mating. Thus, a strain produced by two backcrosses (N3, equivalent to F6) followed by 14 generations of b x s mating (F14) would be fully inbred.

A.1.2.4 Co-isogenic, congenic, and segregating inbred strains

Two strains that are genetically identical (i.e. isogenic), except for a difference at a single locus, are called co-isogenic strains. True co-isogenicity can probably be achieved only by mutation within an existing inbred strain, while lines obtained by inbreeding with forced heterozygosity (segregating inbred), or by backcrossing to an inbred strain (congenic), usually differ in a short chromosomal segment rather than a single gene. The following strains can be formed in accordance with the mutation:

- a) Co-isogenic strains are formed by the occurrence of a mutation within an inbred strain. The co-isogenic strain is separated into a mutant-bearing subline and is precisely defined.
- b) Congenic strains are produced by repeated backcrosses to an inbred strain. (Backcrossing of a mutation onto an inbred strain). Congenic lines that differ at a histocompatibility locus and therefore resist each other's grafts are called congenic resistant (CR) lines.
- c) Segregating inbred strains are developed by inbreeding with forced heterozygosity. Mutation in outbred stock bred in F20 b x s with forced segregation at the mutant locus.
- d) Consomic strains are produced by repeated backcrossing of a whole chromosome such as the X chromosome or Y chromosome onto an inbred strain.
- e) Conplastic strains are developed by backcrossing the nuclear genome from one strain into the cytoplasm of another, i.e. the mitochondrial parent is always the female parent during the backcrossing programme.

A.1.2.5 Hybrids

F1 hybrids are the first generation cross between two inbred strains. They possess all the properties of inbred parental strains except homozygosity. Significant characteristics are being vigorous (less sensitive to environmental influences), and the ability to develop conditions not found in parent strains and to accept tissue transplants from parental strains and from each other. Certain strain combinations are used for development of specific alloantisera. They should not be considered genetically identical. F1 hybrids are designated by listing the female progenitor first and the male progenitor second. The symbol can be abbreviated using standard strain abbreviations.

A.1.2.6 Outbred stocks

To distinguish from standard inbred strains, there is a need for standard ways to refer to laboratory mice in general and to outbred stocks.

- a) **Laboratory mice.** Since laboratory strains are neither pure *Mus domesticus* nor *Mus musculus*, they should be referred to with the words "laboratory mice" or by the inbred strain name when known.

- b) **Outbred stocks.** Outbred or random-bred stocks are sometimes given specific designations if they meet specific criteria. Stock designations shall not be the same as those for inbred strains of the same species.

A.1.2.7 Advanced intercross lines (AIL)

AIL are bred by producing an F2 generation between two inbred strains and then, in each subsequent generation, inter-crossing mice but avoiding sibling matings. The purpose is to increase the possibility of tightly linked genes recombining.

A.1.2.8 Genetically engineered animals

The number of genetically engineered mice available for study is rapidly increasing. Considerable savings in animal life and research funds are possible when existing transgenic or gene-targeted mouse strains can be used for research studies. These animals are used to study gene function, gene expression and gene regulation, to develop animal models of human disease (cystic fibrosis, Huntington's disease, cancer, muscular dystrophy and arthritis), to test gene therapy reagents, to establish cell lines from specific cell types transformed *in vivo*, to produce mice with tissue-specific inducible gene expression or tissue-specific gene deletions, or to study the effects of cell-specific ablation with toxigenes. Examples of genetically engineered mice are:

a) Transgenic mice

A transgenic mouse has a transgene in addition to its normal complement of genes. A transgene is an artificial gene cloned in the lab by recombinant DNA technology and microinjected into fertilized mouse eggs. Eggs are transferred into foster mothers for gestation. Transgenic progeny (founders) are bred to produce a line. Transgenes integrate randomly into chromosomal DNA and are transmitted as a Mendelian trait.

b) Gene-targeted mice

A gene-targeted mouse is derived from an embryonic stem (ES) cell. ES cells are manipulated in culture by introducing a targeting vector that is cloned in the laboratory by recombinant DNA technology. The targeting vector DNA precisely replaces a segment of chromosomal DNA (hence the name "gene targeting") in the ES cell. ES cells are injected into a normal mouse's blastocyst where they mingle with the embryo's cells to form the developing mouse. Up to 100 % of the resulting mouse chimera can be formed from cells descended from the ES cells. ES cell-derived-mouse chimeras are bred to normal mice to produce progeny carrying the targeted gene which is transmitted as a Mendelian trait.

NOTE Chimera refers to an individual whose body contains cell populations derived from different zygotes of the same or different species.

A.2 Defining the microbiological status of animals (microbiological assessment of the health status)

A.2.1 General

General categories used to describe the microbiological status of laboratory animals are gnotobiotic, specific pathogen-free (SPF), and conventional.

A.2.2 Gnotobiotic

Gnotobiotic (Gr. gnotos + biota = known + flora & fauna, and gnotobiote or gnotobiotic animal refer to an animal stock or strain continuously maintained with germ-free techniques in which the composition of any associated flora or fauna, if present, is fully defined by accepted current

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methodology) animals are maintained in isolators and are either germ-free or have a known flora and fauna. There are two types of gnotobiotics:

a) Axenic (or germ-free) animals

Axenic animals are devoid of all other life forms. They are not commonly used, are difficult to maintain and their health monitoring is very intensive. Effects of the germ-free state include enlarged cecum, often resulting in cecal volvulus (up to 15 % death rate), loss of "Colonization Resistance" under stimulated immune response as a result of no microbial antigens present, slower peristalsis, higher intestinal enzyme levels, lower oxygen consumption, and smaller hearts and livers. In order to reverse the latter "negative" effects, these germ-free animals are associated with known strains (usually 8) of bacteria, known as the "Altered Schaedler Flora" (ASF). These germ-free animals will then have a defined flora.

b) Defined flora animal

A gnotobiotic maintained in intentional association with one or more known types of micro-organisms (or any other organism).

A.2.3 Specific pathogen-free (SPF) animals

SPF animals are free of at least one organism. An SPF animal can be free of any number of specified organisms. The amount of health monitoring is therefore dependent on what organisms need to be excluded.

A.2.4 Conventional animals

Conventional animals are all animals not falling into one of the categories in A.2.2 and A.2.3.

Annex B

(informative)

Care and management of laboratory animals — Amphibians (Frogs (*Xenopus laevis*))

B.1 General

Amphibians are scaleless, smooth skinned, ectothermic vertebrates (cold-blooded and their body temperature is dependent on ambient conditions), most of which are closely associated with aquatic or very moist environments.

Virtually all amphibians begin their lives in water as fully aquatic gill-breathing larvae (tadpoles). Some can remain aquatic all their lives whilst others metamorphose into air-breathing adults with lungs and appendages.

The types of amphibians generally encountered in laboratories are frogs, toads and salamanders.

Many amphibians tend to be cannibalistic. Large tadpoles can eat smaller ones and adults can eat tadpoles. High housing density and the mixing of adults and larvae is usually the reason for cannibalism.

B.2 Background information

B.2.1 Range and habitat

The natural habitat for these animals is static or stagnant murky ponds, wells or dams with a substrate of deep mud. Distribution is widespread south of the Sahara. They are able to migrate overland during the wet season.

B.2.2 Species characteristics and biological information

Xenopus laevis is commonly known as the South African clawed frog owing to the presence of small black curved claws on the inner three toes of the hind feet. They are naturally nocturnal, hardy, tailless, stout-bodied, fully aquatic frogs with a long lifespan of 3 years to 20 years in captivity. Their slimy protective skin coating assists in keeping them healthy, and protects them against osmotic changes. The smaller forelegs are used to push food into the mouth.

These animals have very sensitive nerve endings along the body (lateral line) and therefore need to be handled gently.

Adult snout to vent length in two to three year old frogs is 9 cm to 15 cm for females and 7 cm to 9 cm for males (much smaller than females).

The heart rate is 40 beats to 60 beats per minute at a water temperature of 25 °C. The heart is three-chambered with no diaphragm present.

Large fat bodies are attached to each kidney to provide energy during hibernation and reproduction.

Dorsal lymph sacs are paired lymph hearts located dorsally on either side of the last vertebrae (there are 10 vertebrae and ribs are reduced or absent). Intravenous injections are administered via dorsal lymph sacs.

Frogs readily regenerate lost limbs and toes.

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Eggs are spawned and fertilized externally in the spring. Gilled tadpoles develop into lung breathing, tailless four-legged juveniles by 10 weeks to 14 weeks of age. Adults reach maturity at approximately nine months, with sexual maturity peaking at 2 years to 3 years. Development follows a "frog-spawn-tadpole-froglet" transition before they metamorphose into adults. Frog tadpoles are quite large and might need to be re-accommodated several times before they metamorphose.

Sexual dimorphism in non-breeding pairs size is the most obvious difference. Females have much larger ventral flaps (anal papillae) located immediately above the cloaca. Males have black, spinulose nuptial pads on the inner arms and enlarged thumbs to hold onto females during the mating season.

Teeth are present in the upper jaw for gripping food items. In frogs, the tongue is attached to the floor of the mouth instead of being attached to the front of the mouth and is folded back.

Their diet is mainly carnivorous as they eat carrion, small worms, insects, and each other.

Frogs require warm still water and they spend most of their time lying motionless beneath the surface. They are sensitive to sudden changes in water temperature and a sudden variation of 5 °C can kill them. Adults are lung breathers and must come to the surface to breathe or they will drown.

B.2.3 Use in research, testing and teaching

The use of frogs is mainly for genetic, physiological, neurological or endocrine studies, cellular and molecular biology, ecological pollutant and toxicological surveys, and for secondary and tertiary level teaching purposes, including dissection.

B.3 Supply and transport

B.3.1 Procurement of amphibians

Many amphibians are caught in the wild, but purpose-bred animals are preferred for laboratory use because of animal health and welfare reasons, their scientific reliability, and other ethical reasons.

B.3.2 Transport

B.3.2.1 Adult frogs are usually best transported in sealed containers with moist foam cubes to prevent desiccation and to cushion all bumps or shocks. Animals should be of similar size and weight and should not be overcrowded. Shipment containers should provide appropriate constant temperature, separation space, oxygen, and contain potable clean water.

B.3.2.2 If frogs arrive in water, the container should be allowed to adjust to the ambient temperature of the new enclosure. Shipment water may be kept and a gradual changeover to facility water be made by slow dilution. Foam cubes and other solid items should be discarded.

B.3.2.3 For frogs being transported by air, the IATA standards and recommendations are to be adhered to (see 7.3.2). Flights should be planned to ensure the journey times are of the minimum duration possible.

B.3.2.4 Communication between supplier, recipient, handling agents and border control officials is essential to eliminate delays and animal welfare issues.

B.3.3 Quarantine

B.3.3.1 An appropriately trained person should be on hand to receive and conduct health checks on new arrivals.

B.3.3.2 All frogs should be quarantined for 30 d on arrival at a new facility. A quarantine period of 90 d should be considered for frogs caught in the wild.

B.3.3.3 New introductions should be handled last during the daily routine management. Frequent and appropriate handwashing procedures and glove changes are essential.

B.3.3.4 Quarantine may be done in the same room but separate tanks or cages should be used. All equipment, gloves, etc. shall be designated to the specific tank(s) use only. Every precaution should be taken to avoid any cross-contamination to other healthy residents.

B.3.3.5 New arrivals should be monitored at least twice daily for any changes in health status. Preventative treatments for any newly shipped frogs may include placing them in a 0,6 % calcium hypochlorite solution (or a 0,06 % sodium chloride solution) to reduce the growth of *Pseudomonas spp.*, *Proteus spp.*, *Aeromonas spp.*, and the occurrence of "red leg".

B.4 Housing and care

B.4.1 Humane handling

B.4.1.1 When frogs are being moved for cage or tank changes and for cleaning or manipulations, use of a net is recommended to catch and handle the frog. A hand should be placed over the top of the net to prevent the frog from jumping out. Nets (sufficiently deep, strong and non-abrasive) are less traumatic than hand-catching and also help reduce body contact.

B.4.1.2 When restrained out of water for any length of time, handling should be done gently, and frogs kept moist. Both hands should be used to form a closed cup, as opposed to applying pressure to physically grip the animal. Unnecessary movement of objects or hands over the tank should be avoided.

B.4.1.3 Personnel should be well informed and trained regarding any pain and distress frogs might experience as a result of experimental procedures. They should be aware of responses frogs are likely to show, what the humane endpoints of any project are, and what actions to be taken in the event of unexpected developments.

B.4.2 Lighting

B.4.2.1 Frogs should ideally be kept in a room with no windows or all external lighting cues should be blocked out. Lights (fluorescent) should simulate outdoor conditions with UV to maintain vitamin D levels.

NOTE A 12 h/12 h light/dark cycle is recommended.

B.4.2.2 Continuous light, light deprivation, and inappropriate photoperiods can cause varying symptoms in the frogs ranging from lethargy to sterility. Where artificial lighting is used, it is recommended that gradual brightening and dimming periods of 30 min be used in the morning and in the evening.

B.4.2.3 Frogs should not be subjected to bright light, but light must be adequate for inspection purposes. Dimmer systems are recommended. Darkened refugia should be provided. Disturbances

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for cleaning, feeding and other regimes should be avoided and management planned to cater for the frog's nocturnal behaviour, accordingly.

NOTE Any artificial light used should simulate outdoor sunlight levels.

B.4.3 Thermoregulation

B.4.3.1 Since amphibians are ectotherms, they cannot raise their body temperature by producing metabolic heat. All species have a preferred body temperature range at which they function optimally.

B.4.3.2 Tadpoles prefer slightly warmer temperatures. Enclosures and tanks should be able to provide a range in temperature (heat tape, heating pads and heat lamps). This allows the tadpoles to move within the range of temperature and regulate their own body temperatures.

NOTE The thermal critical maximum temperature for *Anura* is 35 °C.

B.4.4 Water provision and quality

B.4.4.1 The volume and depth of water is largely determined by frog size and should increase proportionately for larger frogs to allow normal behaviour, including "serenading" (standing on hind legs and chirping at night) (see 7.6.1.2).

B.4.4.2 Water should be clean and potable, and treated to remove all chlorine or chloramines. Chemicals to remove chlorine or chloramines from tap water are available commercially. Amphibian skin is more porous than most other vertebrates and this makes them very sensitive to toxic substances (fluorides, chlorine, chloramines and heavy metals), micro-organisms in the water, and changes in the pH value and oxygen levels.

B.4.4.3 *Xenopus spp.* water temperature should be in the range of 20 °C to 25 °C. Breeding can decline at warmer temperatures. They are also sensitive to temperature changes in the holding room. When changing the water, temperature variations should not be allowed to exceed 2 °C. Greater sudden variations can induce shock. Suboptimal water temperatures cause metabolic and immune systems depression, and adversely affect appetite and reproduction.

B.4.4.4 Frogs routinely shed skin particles, and release faeces and ammonia wastes into the water. Water should be changed daily or at least on alternate days, usually about 2 h to 5 h after feeding. Do not use distilled water.

B.4.4.5 Personnel changing the water should wear gloves. Traces of hand lotions, colognes and medicated ointments will affect or kill frogs. Smoking in frog holding rooms is not permitted.

B.4.4.6 Water quality can be affected by location, supply and source, quantity, treatments, type of foods fed and amounts accumulating in the water. Fresh water out of the tap is oversaturated with gases and will cause bubbles under the skin and in the toe webs of frogs. Tap water should be allowed to stand for at least 2 h to 6 h before use.

Water pH values above or below the range of pH 6,5 to pH 8,5 can cause sudden deaths in a colony. Higher pH values increase toxicity of chemicals in the water, especially ammonia.

The guidelines in table B.1 may be used for water quality control.

Table B.1 — Guidelines for water quality control

1	2
Determinands	Allowable quantity
Alkalinity and hardness as CaCO ₃	50mg/L to 150 mg/L
Ammonia	< 0,2 mg/L
Nitrates	< 0,5 mg/L
Dissolved gases/carbon dioxide	< 5 mg CO ₂ /L
Chlorine/chloramine	< 3,8 mg/L
Fluorides	< 1,5 mg/L
Heavy metals (zinc, copper, mercury and lead)	nil
Toxicants from insecticides or other sources	< 80 % saturation
Oxygen (less than 80 % saturation): warm water	> 5 mg/L
cold water	> 8 mg/L

Water samples can be tested periodically at a laboratory to ensure constant quality.

B.4.4.7 Avoid galvanized or copper piping. Zinc may be leached from galvanized pipes and copper from copper or brass pipes.

B.4.5 Tank housing

B.4.5.1 Tanks used are generally of the following two types:

- a) **Standing (static) water system** – periodically emptied and refilled daily or on alternate days. This is a better option for disease control.
- b) **Drip through system** – continuous, slow and regular water changes. Toxic waste levels are kept continually low. Frogs do not like strong circulating water or currents. Flow rate should not exceed 10 mL/min.

B.4.5.2 Opaque aquaria are optimal. Black backgrounds are preferred. Bottoms of the tanks should be completely lightproof and dark to simulate the dark lower pond levels.

B.4.5.3 Glass, fibreglass, polycarbonate, plastics (of human food storage quality) and stainless steel aquaria will require adequate refugia.

NOTE Tank materials should not add toxic substances to the water.

B.4.5.4 Since frogs can jump out of aquaria, all tanks need to be lidded or screened. Frogs which escape will dehydrate and die.

B.4.5.5 Each tank should be clearly identified and have a record card with all data on frogs housed therein, experimental details and any treatments being given. All animal welfare issues should also be recorded here.

B.4.6 Sanitation and disinfection

B.4.6.1 Ideally, tanks should be cleaned daily or at least three times per week. Regurgitation of food by frogs can occur if they are disturbed too soon after eating. A period of 3 h to 5 h should be allowed to elapse after feeding and before disturbing the frogs. Regular water changes are necessary to remove uneaten putrefying food.

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B.4.6.2 Frogs are very susceptible to intoxication by phenol and cresol type disinfectants. These will cause convulsions, flaccid paralysis and rapid death. Tadpoles are extremely sensitive to chemicals.

A 10 % bleach or iodine scrub are the disinfectants of choice. Thorough rinsing of the tanks is still required after washing. If a cage washing machine is used, cages should be put through a double rinse cycle, or be hand rinsed again.

B.4.7 Identification methods and records

B.4.7.1 The actual need for individual identification needs to be justified, and only the most non-invasive and humane method(s) should be used.

B.4.7.2 Amphibians normally shed skin, therefore tattooing or branding is ineffective as they soon become illegible. Frogs can regenerate toes therefore the toe clipping method is unacceptable. Plastics legs bands slide off due to the slimy skin secretions, and placing them too tightly on the leg will cause circulatory disturbances and necrosis.

Preferred methods of identification are photographic records as well as skin pattern diagrams.

B.4.8 Group housing

B.4.8.1 It is preferable that frogs be housed in groups as they naturally form hierarchies in territorial habitats. If group structures are altered regularly or groups are too big, then antagonistic behaviour will be evident.

B.4.8.2 Frogs are easily housed in a tank with chlorine-free and chloramine-free water to a depth of 8 cm to 10 cm. Standard polycarbonate rat cages (48 cm x 27 cm x 20 cm) with a lid will hold 10 L of water. This is adequate for four to five frogs. It is preferable to house less per tank if all are female. Frogs should be segregated by size to prevent cannibalism, and by sex unless breeding is the aim.

On average, groups of five to twenty frogs per tank are held in the laboratory.

B.5 Handling techniques

B.5.1 As potential prey species, frogs do not like being held or restrained. The skin is highly glandular (mucous secreting) and is easily damaged. The lateral line sensory system is highly sensitive. Therefore, repeated disturbance for capture and handling should be reduced to a necessary minimum.

B.5.2 Latex gloves should be worn. This will protect the handler from harmful infectious agents in the water and will protect the slimy skin of the frogs. It is important not to allow frog skin or parotid gland secretions to come into contact with the eyes as they are extremely irritant with painful side effects.

Powdered gloves should not be used. Gloves should be moistened with water before handling frogs.

B.5.3 Frogs jump forward and dart backwards. It usually takes two hands to hold them as they will not go limp and easily allow single hand restraint.

B.6 Nutrition (Food types and feeding regime)

B.6.1 Frogs are carnivorous and eat submerged in the water. They should be fed two to three times per week. Commercially prepared pellets, and those designed for carnivorous fish, are a balanced and complete diet. They also eat small worms, meal worms, crickets, grubs, or small pieces of raw beef heart or liver (which should not be fed as the sole diet).

B.6.2 Feeding frogs at the end of the day is recommended as they consume and digest their food undisturbed during the night and chances of regurgitation are greatly reduced.

NOTE Frogs can become quite tame and will swim to the surface at feeding time and often accept food from the feeder's hand.

B.6.3 Frogs feed in a frenzy and will devour food within minutes. Allow 2 h to 5 h for complete feeding. If food remains after this period then too much is being given.

Attacking each other at feeding time usually indicates overcrowding or frogs being underfed.

B.6.4 It is important to watch animals eating. Those not feeding might be sick and should be removed from the tank.

B.6.5 Frog tadpoles are herbivores and filter feeders. They will begin feeding on suspended food particles in about 10 d. They will hang, head down, at a 45° angle in mid-water, with tails vibrating. This causes a current which draws food towards them. Water is drawn in through the mouth and expelled through the gills after the food particles have been filtered out. Strained baby food (green beans or peas) at 1 drop per 100 mL water for 25 tadpoles can be used. Overfeeding should be avoided, and this can be monitored by the cloudiness of the water from previous feeds.

B.6.6 Tadpoles have a high calcium requirement and, in addition to the food source, will absorb calcium through the gills and skin. Iodine deficiency can cause tadpoles to fail to metamorphose.

B.6.7 Frogs and other *anura* do not drink but absorb water through a highly permeable specialized area in the pelvic region.

B.7 Environmental enrichment

B.7.1 Frogs require refugia in the form of PVC pipes, aquaria rocks and other items they can seek cover under. They will ingest small objects such as beads, gravel, or marbles so these should not be put into tanks. Enrichment structures and items shall have smooth and rounded edges to prevent injury.

B.7.2 Human food grade containers can be used as refugia "caves" for frogs. Any objects placed or floating on the water surface should not prevent the frogs from coming up to the surface to breathe. Rest areas on the water surface could be required depending on species habitat requirements.

B.7.3 Gravel substrate should not be used since frogs are natural mud dwellers (see B.2.1).

B.8 Health assessment and disease prevention

B.8.1 A frog's welfare can be compromised by poor or ill health. Prevention of disease in frogs is easier than treating sick animals.

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Frogs are hardy animals and rarely get sick. They can carry a range of pathogens without the development of disease until there is a disturbance in physiology owing to environmental stresses. Environmental disturbances, such as poor water quality, can cause predisposition to illness.

B.8.2 Signs of illness are postural changes, diminished avoidance responses and righting reflexes, lethargy and slow movements, hanging out on the water surface, inability to dive, staying at the bottom of the tank, bloating, shedding large amounts of skin, white fluffy cotton-like growths on the skin, and reddened body appendage(s). Such animals should be removed from the tank and isolated.

B.8.3 Body condition should be evaluated by looking at the prominence of the skeleton and abdominal contents. Body weight is highly variable and often depends on the state of hydration. Frogs can lose up to 50 % of body weight in fluids before death.

B.8.4 Skin scrapings and gill biopsies are useful to detect fungal, bacterial, and parasitic infections. Faecal examinations can detect protozoan and metazoan parasites. Radiology, fibreoptics and trans-illumination are other useful diagnostic resources.

B.9 Common diseases

B.9.1 Bacterial diseases

B.9.1.1 Bacterial diseases are the principal causes of death in laboratory amphibians. The agents are usually normal flora of amphibian environments which invade and become pathogenic after the animal's immune system is compromised. Most are gram-negative such as *Pseudomonas spp.*, *Proteus spp.*, *Aeromonas spp.* and *Acinetobacter spp.*. *Salmonella spp.* is frequently isolated from the faeces of healthy *anura*, but can be pathogenic.

B.9.1.2 Some bacterial diseases that amphibians are prone to are:

- a) "Red leg" – This is a bacterial septicaemia which causes high morbidity and mortality in frogs. It is caused mainly by *Aeromonas spp.*, *Proteus spp.* and *Pseudomonas spp.* along with other contributing bacteria. By the time frogs show clinical symptoms, the condition is usually terminal. This disease can spread rapidly through a colony.
- b) Tuberculosis – This is caused by *Mycobacterium spp.* and probably gains entry via skin wounds and abrasions. Most immunocompromised animals are affected. Granulomas that develop in major organs lead to progressive debilitation.

B.9.1.3 It is recommended that cultures be taken regularly for effective antibiotic treatment.

B.9.2 Fungal diseases

Fungal diseases are common in amphibians and are usually secondary to stress, trauma, and poor hygiene. Granulomas or abscesses can be found in any organ. Skin ulcers and nodules are common signs. Infections often recur after antifungal treatment is completed.

B.9.3 Parasitic diseases

Amphibians have a large number of parasites which generally do not cause problems. Quarantine procedures should include routine anthelmintic treatments.

Nematodes, such as strongyloid lung worms, can cause pneumonia and poor growth. Adult worms can be found in the lungs, eggs and larvae, and in large numbers in the gut canal, coelomic cavity and lymph spaces.

B.9.4 Protozoans

Aquatic species are susceptible to infection by skin protozoa which cause skin irritation, cloudiness, and excessive mucous production.

B.9.5 Non-infectious diseases

B.9.5.1 Neoplasia occurs but is not common.

B.9.5.2 Rectal and cloacal prolapses are not uncommon and are usually secondary to ascites. The affected recta and cloaca can be replaced, but most recover spontaneously.

B.9.5.3 Dehydration, chemical toxicities, water supersaturation with air, trauma, poor hygiene, etc. all cause a predisposition to various disease conditions.

B.10 Recommended doses of therapeutic agents for amphibians (see table B.2)

Dose rates vary with temperature, renal function, hydration state, and ambient humidity. Percutaneous therapy can be useful because of amphibian skin permeability, and can be used for rehydration purposes via misting.

Anaesthetics, antibiotics and anthelmintics can be effectively administered when used in the water or dropped onto the skin.

Table B.2 — Recommended doses of therapeutic agents for amphibians

1	2	3
Drug	Recommended dose ^a	Recommended route and dose interval ^a
Amikacin	5 mg/kg	SC, IM, IP, q 24 h
Benzalkonium chloride	0,25 mg/L 2 mg/L	Bath for 72 h Dip 1 h, q 24 h
Carbenicillin	200 mg/kg	SC, IM, IP, q 24 h
Chloramphenicol	50 mg/kg 20 mg/L	Bath SC, IM, IP, q 24 h Bath
Enrofloxacin	5 mg/kg	SC, IM, IP, q 24 h
Gentamycin	2,5 mg/kg to 5 mg/kg 10 mg/L	Bath SC, IM, IP, q 24 h Bath
Ketoconazole	10 mg/kg	PO, q 24 h
Methylene blue	4 mg/L	Bath
Nalidixic acid	10 mg/L	Bath
Nitrofurazone	10 g/L to 20 g/L	Bath
Sodium chloride	4 g/L to 6 g/L	Bath for 72 h
Sulfamezathine	1 gm/L	Bath
Oxytetracycline	50 mg/kg 25 mg/kg 1 g/kg diet	PO, q 12 h SC, IM, q 24 h 7 d
Trimethoprin	3 mg/kg	SC, PO, q 24 h

^a To be prescribed by a qualified veterinarian.

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B.11 Antiparasitics (see table B.3)

Table B.3 — Recommended doses of antiparasitics for amphibians

1	2	3
Drug	Recommended dose ^a	Recommended route and dose interval ^a
Copper sulfate	500 mg/L	Dip 2 min, q 24 h
Formalin 10 %	1,5 mL/L	Dip 10 min, q 48 h
Ivomectin	0,2 mg/kg to 0,4 mg/kg	Percutaneous as prescribed
Levamisole	300 mg/L	Bath for 24 h
Metronidazole	10 mg/kg to 40 mg/kg every 24 h	PO 5 d, repeat as needed PO 5 d
Sodium chloride	4 g/L to 6 g/L to 25 g/L	Bath or dip for 10 min
^a To be prescribed by a qualified veterinarian.		

B.12 Scientific procedures

B.12.1 General

B.12.1.1 There are a number of procedures commonly carried out on frogs in order to obtain and rear their eggs. These include egg-harvesting, oocyte collection and induction of anaesthesia. All these have the potential to cause pain, distress or suffering.

B.12.1.2 The main techniques used for the procurement of eggs are:

- a) natural mating;
- b) induction of ovulation; and
- c) "squeezing" of females (only to be performed by experienced personnel).

B.12.2 Breeding and reproduction

B.12.2.1 Amphibians fertilize eggs externally during amplexus (males release sperm over freshly laid eggs) via cloaca release. This process can last up to 12 h or more. The eggs are single-celled and jelly-coated, are laid in the water and are very sensitive to dehydration, and thus require a high humidity to be viable.

B.12.2.2 Breeding activity might be preceded by hibernation and is usually triggered by environmental conditions such as rainfall, increased day length (photoperiod), increased temperature and humidity, and abundance of food.

B.12.2.3 In captivity, frogs can be bred by manipulation of their environment to simulate breeding conditions. Temperature, photoperiod and humidity can be lowered and then raised again. Misting can be used to increase humidity.

In frogs, breeding can be artificially induced by hormone manipulation using gonadotrophic hormone injections, at any time of the year. Pregnant Mare Serum Gonadotrophin (PMSG), Human Chorionic Gonadotrophin (HCG), or Gonadotrophic Releasing Hormone (GnRH) may be used. For

mating or release of sperm to occur, males will need to be injected with HCG or GnRH. Frogs generally receive an initial dose followed by a second hormone dose 8 h to 36 h later. Frogs should be paired after the second dose and left undisturbed in a darkened breeding tank until mating is over.

B.12.2.4 Amphibian ova require a long formation, development and maturation process (oogenesis) which depends heavily on the nutritional status of the female. Stress is a powerful inhibitor of amphibian reproduction.

Females should be bred no more than once per month. Females that have not bred for four to six months tend to deposit an increased number of necrotic eggs.

The expected breeding life of the female frogs is one to two years. Males in good health have a breeding life of three years or slightly longer, but they should not be bred more than two to three times per month.

B.12.3 Egg-harvesting and care

B.12.3.1 General

During egg development, the water must be chlorine- and chloramine-free, and gently aerated. A pH value of 6,5 to 7,5 and a temperature of 20 °C to 23 °C is recommended.

B.12.3.2 Oocyte collection

B.12.3.2.1 Oocytes are immature eggs not yet capable of being fertilized. Collection purposes are primarily for research in molecular biology, biophysics, ontogenesis and genetics.

B.12.3.2.2 Surgical methods are much more invasive than the process of egg collection and require the removal of ovarian tissue under anaesthesia. Repeated episodes of surgical removal are not acceptable. If oocytes are to be taken from a frog on more than one occasion, the second collection should be under terminal anaesthesia.

B.12.3.2.3 It is essential to recognize that frogs need and should be given appropriate perioperative pain relief.

B.12.3.2.4 Skin sutures and clips should be removed after two to three weeks, and good post-operative care is essential.

B.12.4 Hatching larvae or oocytes

The hatching larvae or oocytes generally stay attached to the jelly while the yolk sacs are absorbed. Feeding will commence in 5 d to 10 d.

Tanks should be kept clean and aerated.

B.12.5 Care and rearing of tadpoles

B.12.5.1 Tadpoles should never be placed in chlorinated water. When small, they may be kept in 50 L artificial pond water, as formulated in table B.4, or in 5 L when metamorphosis begins. For best results, 50 % of the water should be changed daily.

B.12.5.2 To provide artificial pond water, 20 mL solution A + 20 mL solution B (see table B.4) + 5 mL distilled water should be mixed.

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Table B.4 — Pond water formula

1	2
Stock solution A	Stock solution B
175 g NaCl	5 g NaHCO_3
35 g CaCl_2	
2 L distilled water	2 L distilled water

B.12.5.3 After metamorphosis, froglets receive the same treatment as adults.

B.12.5.4 All dead or diseased frogs should be removed as soon as possible from larval stage tanks.

B.12.5.5 Handling tadpoles is best done using scooping containers and not nets, to avoid damage to the skin and gills.

B.12.6 Hibernation

B.12.6.1 Hibernating frogs should not be fed.

B.12.6.2 Hibernation is achieved if the temperature is lowered gradually over several days, with a 3 °C to 5 °C drop per day, until the hibernation temperature for the specific amphibian is reached. Frogs will hibernate when the temperature drops below 8 °C.

B.12.6.3 Rapid hibernation, hibernation out of season or hibernating the wrong species at the wrong temperature will cause shock and death.

B.13 Blood collection

B.13.1 Blood collection in amphibians is difficult owing to low body temperatures and poor access to vessels. Maximum blood sample volumes can only be obtained by euthanasia.

B.13.2 Blood collection is carried out via clipping of the toe web, cardiac puncture, or venous cuts of ventral abdominal veins. Cardiac puncture and venous cuts as terminal procedures should be performed on anaesthetized animals only, and death should be confirmed after collection. Toe clipping requires the use of local anaesthetic.

B.14 Injections and sites

B.14.1 The ideal needle gauge for most amphibians has a weight of 25G to 27G and a length of 1cm to 1,5 cm. The syringe with any injection should always be aspirated, and thereafter be sited.

B.14.2 Drugs can be administered via the dorsal lymph sacs where they are rapidly absorbed. This is the common site used for hormone injections.

The thigh muscles can be used for intramuscular injections.

In intra-peritoneal injections, where the needle is inserted into the groin area, the frog should be on its back in the handler's hand, with its head directed downwards.

B.14.3 Gavage by stomach tube is very stressful to amphibians and is not recommended.

B.15 Analgesia and anaesthesia

B.15.1 Delicate species, including *Xenopus*, should be handled with soft nets for any non-anaesthetized minor procedure. Chemical restraint is required for all prolonged or invasive procedures.

B.15.2 Fasting of frogs before anaesthesia, at the recommended dose as given in table B.5, is recommended.

B.15.3 Anaesthesia is judged by the loss of righting reflexes and respiratory effort. As the anaesthesia deepens, abdominal respiration is lost followed by the slowing of the throat movements which stop when surgical level is reached. At low temperatures, cutaneous respiration appears to provide oxygen to support life. The frog should be kept moist under anaesthesia so that percutaneous respiration is not interrupted.

B.15.4 Frogs should not be returned to tanks and placed in water until fully recovered. They may be propped on wet tissue or foam rubber.

Table B.5 — Anaesthetic doses for amphibians

1	2	3
Anaesthetic ^a	Amphibian type	Dose
MS-222 (bath)	Tadpoles, frogs, toads, newts and salamanders	300 mg/L to 500 mg/L to effect buffer with NaHCO ₃
MS-222 (inject)	All	50 mg/kg to 150 mg/kg subcutaneous, intramuscular
Benzocaine	Larvae, frogs and salamanders	50 mg/L dissolved in ethanol 200 mg/L to 300 mg/L
Ketamine	All	50m g/kg to 150 mg/kg subcutaneous, intramuscular
Isoflurane Halothane	Terrestrial species	4 % to 5 % in anaesthetic chamber
^a Ketamine and zoletil can be used for minor procedures such as radiography. Animals anaesthetized with these drugs still feel pain, even at high doses.		

B.16 Euthanasia

B.16.1 Euthanasia is most easily accomplished through use of an overdose of MS-222 by immersion. It may also be given by intravenous (IV) or intraperitoneal (IP) injection.

Sodium pentobarbital (60 mg/kg to 100 mg/kg) is injected into the dorsal lymph sac or IP.

B.16.2 Pithing, a process of decerebration followed by spinalization, is not acceptable. Decapitation SHALL be preceded by chemical restraint (as given in B.16.1).

B.16.3 Other unacceptable methods include freezing (hypothermia), and the use of formaldehyde solution, carbon dioxide, ether, chloroform, exsanguinations without anaesthesia, chloral hydrate, ketamine HCL, and chlobutanol.

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B.17 Training of personnel

Personnel working with and responsible for the care and welfare of amphibians should be adequately trained and should have a good understanding of the animals they are involved with.

Inappropriate handling will impact on the animals' wellbeing and affect scientific results. Training of personnel should be species-specific.

B.18 References

See bibliography.

Annex C

(informative)

Care and management of laboratory animals — Birds

C.1 General

C.1.1 All birds are essentially built for flight and therefore share the same basic anatomy, despite their diverse range of adaptations for locomotion and feeding.

C.1.2 Most species of birds are highly social for at least part of the year, and should be kept in stable groups wherever possible. They are highly sensitive to family relationships and the formation of appropriate, stable, and harmonious groups. They have a strong capacity for social learning from watching the behaviour (foraging and feeding) of parents or others.

C.1.3 Bird behaviour, physiology and ecology are variable and diverse. Substantial suffering can be caused if housing and care are inappropriate, and this is unacceptable for ethical and scientific reasons.

C.1.4 The design of housing and care systems should allow for:

- a) identification of behavioural requirements and design protocols that stimulate the range of natural behaviour;
- b) simulation of appropriate wild conditions;
- c) inclusion of compatible conspecifics for social species, and appropriate sex ratios;
- d) provision of sufficient space for exercise and roosting;
- e) encouragement of foraging behaviour; and
- f) promotion of good health and welfare.

C.2 Use of birds in research

Birds are used for a wide variety of purposes including fundamental research, applied veterinary and medical studies, ecological studies, antibody production, pharmacological safety and efficacy testing, toxicology studies, and animal welfare studies (especially where birds are kept under intensive systems). The following are important considerations:

- a) The capture of wild birds for research should be avoided unless soundly justified.
- b) Conservation, Convention on the International Trade in Endangered Species (CITES) of Wild Fauna and Flora (see C.4.2 (d)) and animal welfare legislation should be complied with.
- c) All potential or recognized pain in birds should be appropriately alleviated.
- d) It is assumed that avian cognitive skills are equivalent to those of mammals, and that birds also need a stimulating environment.
- e) Birds can detect changes in sound direction and to avoid startling them, approaches should be quiet but audible. Birds should be protected from excessive or sudden loud noises.

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- f) It shall be ensured that laboratory personnel all wear the same colour protective clothing and that the colour is not changed.
- g) Indoor-housed birds should be protected from odours of mammalian or other predators.
- h) A balanced diet should be provided to those species that eat a diverse range of foods.

C.3 Training of personnel

Adequate and appropriate training of all personnel involved with the care and maintenance of captive birds, in the laboratory or field, is essential, and this should include specialist training in catching and handling, pain recognition, avian nutrition, and study techniques.

C.4 Procurement of eggs or birds

C.4.1 General

C.4.1.1 Whether study birds should be acquired as adults or raised from chicks will depend on the nature and duration of the study, the behavioural characteristics, the conservation status of the species, and the intended fate of the birds (euthanasia, re-homed, or released back into the wild).

C.4.1.2 When purchasing birds from breeders and suppliers, information on the hatching, rearing, and housing conditions as well as their welfare and health status should be obtained.

C.4.1.3 The following are important considerations when acquiring birds:

- a) the welfare costs and benefits associated with rearing from hatch versus obtaining adult birds for a study should be assessed;
- b) the birds' quality of life after procedures have ended, and their eventual fate should be considered;
- c) that birds should be bought from reputable breeders or suppliers; and
- d) that stress should be minimized by introducing changes to environment and husbandry gradually.

C.4.2 Removal of eggs or birds from the wild

The following are important considerations when removing eggs or birds from the wild:

- a) national and international laws regulate the taking of eggs and birds from the wild;
- b) taking eggs or birds from the wild should be avoided unless there is sound ethical justification;
- c) the potential for disrupting the remaining individuals should be minimized;
- d) all CITES and nature conservation legislation should be adhered to;
- e) all necessary licences should have been granted before conducting field studies;
- f) use of endangered or threatened species should be avoided;
- g) removal of eggs from the nest should be done when the nest is unattended, and no more than half the clutch should be removed without sound justification;

- h) trapping of adult birds should all be carried out by experienced personnel;
- i) stress should be reduced by using passive walk-in or fly-in traps where possible;
- j) stress should be minimized by allowing a period of quarantine and adaptation before a study begins;
- k) birds that are kept in bird bags for other than short periods should be held in dark well ventilated areas where they can preen comfortably; and
- l) all field procedures should be carried out under sterile conditions, and appropriate anaesthesia and analgesia should be used.

C.5 Releasing birds after field studies

Before releasing birds after field studies the following should be done:

- a) birds should be carefully examined after procedures for signs of shock, haemorrhage, disability or injury;
- b) it should be ensured that appropriate transport containers, and the means to put to death by recognized euthanasia methods, are readily at hand;
- c) birds should be allowed to move away in their own time, and on release it should be observed that they can walk and fly effectively; and
- d) the effects of external and internal marking methods on wild birds should be considered.

C.6 Transport of birds

The following are important considerations for the transport of birds.

- a) Anyone transporting birds should be aware of all relevant legislation and obtain the necessary permits.
- b) Transport guidelines and standards should be consulted, and specific species requirements should be taken into account. Planning journey routes and time is advisable. Contingency plans should be prepared and trained personnel should be in attendance.
- c) Bird containers should be appropriate for the size and numbers of birds being transported.
- d) Eggs require special containers to prevent breakage, and transporting eggs during the first 2 h to 3 h of incubation should be avoided.
- e) Transported eggs should be insulated, or be transported in an incubator.

C.7 Breeding and rearing birds from hatch

C.7.1 Rearing birds from hatch, either by breeding or purchasing the eggs, might be the preferred option if a study requires repeated close human contact for procedures, or if the birds have to be killed at the end of the study.

C.7.2 Before breeding birds, it should be considered whether there is a sustained requirement to continue with the species, whether appropriate conditions can be sustained long term, and how breeding can be rationalised to prevent over-breeding and waste.

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C.7.3 The following points should be considered when breeding and rearing birds from hatch:

- a) all unnecessary disturbances to breeding birds, without compromising health and welfare should be eliminated;
- b) wherever possible birds should be allowed to hatch and rear chicks themselves;
- c) advice should be obtained on the correct type of incubation systems, and optimum conditions, before acquiring the eggs;
- d) the sterilization of eggshells, incubators and hands before handling and placing eggs should be ensured. If broody birds are used to incubate eggs, it should be ensured that their care and welfare is addressed adequately;
- e) incubators should be set at the correct temperature and humidity for each species. These readings should be monitored and recorded regularly, and problems rectified immediately;
- f) it should be ensured that emergency back-up systems are available;
- g) accurate records should be kept so that development can be monitored and waste minimized;
- h) eggs should be labelled clearly with wax or graphite and should be "candled" regularly to check for non-viable eggs;
- i) eggs from different species should not be incubated in the same machine;
- j) at hatching, it should be ensured that temperature and humidity levels are correct and hatchlings should be checked at least twice daily;
- k) it should be ensured that hatchery floors are non-slippery so that chicks can grip the surface;
- l) growth rates should be checked regularly;
- m) feeding intake should be checked; and
- n) ideally, chicks should be reared in broods or groups of conspecifics and never in isolation, unless soundly justified for scientific reasons.

C.8 Diet for adult and juvenile birds

The following are recommended essentials concerning the diet for birds:

- a) birds should be fed their natural diet wherever possible and supplemented when necessary;
- b) taste and variety should be assumed as being important to some species;
- c) dietary enrichment should be provided when required, but sudden, abrupt diet changes should be avoided;
- d) grit should be supplied in various sizes for birds to choose from; and
- e) it should be ensured that dietary calcium and phosphorus are provided in recommended forms and appropriate levels for each life stage.

C.9 Catching and handling in the laboratory

The following points should be considered when catching and handling birds:

- a) suitable equipment for catching should be available for use. Well-maintained nets of appropriate sizes, with darkened netting and padded rims are recommended;
- b) if study procedures require frequent handling, they should be performed by competently trained personnel. From an animal welfare perspective, it is advisable to handle chicks frequently during rearing since this reduces fear of humans at later stages;
- c) unless the birds have been adequately habituated, excessive handling of birds should be avoided because they find this very stressful and might view human handlers as predators. Care should be taken not to drop or injure the birds;

NOTE Bruising, wing sprain, skin damage and broken bones are common handling injuries.

- d) during handling, respiration should not be impeded by compression of the sternum or kinking of the neck;
- e) induction of hyperthermia (heat stress) should be avoided;
- f) birds should never be caught by the wings or legs alone;
- g) birds should be held securely so that they cannot damage their wings or legs;
- h) small birds should be caught in dim light, and should be approached from behind;
- i) it should be ensured that the room is escape-proof since small birds can escape fairly easily;
- j) large birds should be handled firmly and positively;
- k) flocking birds should be approached slowly and deliberately;
- l) large birds should not be caught by the wings or the wings be interlocked;
- m) leg movements should be restricted and wing flapping prevented in domestic poultry;
- n) birds should not be carried upside-down by the legs;
- o) large birds should be released gently onto the floor;
- p) birds will try to defend themselves and might try to peck handlers therefore handling techniques should be demonstrated to all responsible persons;
- q) the neck should not be grasped forcefully or the airway be obstructed; and
- r) inducement of tonic immobility (freezing) by turning birds onto their back in dorsal recumbency should be avoided. This causes unnecessary stress, is not hypnotic, and since birds are still aware of their surroundings, they will experience pain and fear.

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C.10 Chemical restraint

Chemical restraint might be necessary when handling birds in the laboratory for certain non-invasive or painless procedures. Sedatives should be approved for use, and should be administered by a veterinarian or authorized person. If competent and empathetic handling will achieve the same result, then chemical restraint should not be used.

C.11 Housing and husbandry

C.11.1 The environment

C.11.1.1 It might be necessary to consider a combination of solid and grid flooring for scientific purposes, where the solid section should comprise one third of the total enclosure area. Grid areas should be located under perches if faecal collection is required.

Wire and grid floors do not allow dust bathing, scratching, pecking and foraging, and are considered detrimental to birds' health and welfare as they can cause foot lesions.

C.11.1.2 Birds should be inspected at least twice daily for abnormal behaviours and for any sickness.

C.11.1.3 A comprehensive health plan should be drawn up with a veterinarian before acquiring the birds.

C.11.2 Temperature and relative humidity (see table C.1)

C.11.2.1 Where possible, birds should be provided with a range of temperatures so that they can exercise a degree of choice over their thermal environment.

NOTE Brooders, juveniles or sick birds might need supplementary heat.

C.11.2.2 All adult healthy quail, pigeons, domestic fowl, ducks, geese, and turkeys should be housed at temperatures between 15 °C and 21 °C. It is important to take into account the interaction between temperature and relative humidity as some species will suffer from heat stress within the prescribed range if relative humidity is too high.

C.11.2.3 Chicks of all species should be evenly spread, and should make a moderate amount of noise. Behaviour should be monitored as quiet chicks could be too hot, and noisy distress calls and huddling under the lamp source usually indicate that chicks are too cold.

C.11.2.4 Relative humidity (see table C.1) should be maintained within the range of 50 % to 70 % for healthy adult birds.

Table C.1 — Temperature and relative humidity per age group

1	2	3	4
Age	Under lamp	Ambient room temperature	Relative humidity
days	°C	°C	%
Up to 1	35	25 to 30	70 ± 10
2 to 7	32	22 to 27	70 ± 10
8 to 14	29	19 to 24	40 to 80
15 to 21	26	18 to 21	40 to 80
Over 21	-	15 to 21	40 to 80

C.11.3 Ventilation

C.11.3.1 Many species are very susceptible to draughts and care should be taken not to allow birds (especially juveniles) to become chilled.

C.11.3.2 Accumulation of dust and gases, such as carbon dioxide and ammonia, should be avoided.

C.11.4 Lighting

C.11.4.1 Normal physiological functioning demands show that light quality and quantity are critically important for some species at certain times of the year. Requirements of juveniles will vary from those of adults.

C.11.4.2 Lights should not be abruptly switched on or off, but dimmed or raised in gradual intensity. Dim "night lights" can be provided. The avian retina is considerably more complex than that of humans, and birds (and especially raptors) have excellent colour vision.

C.11.5 Noise

Birds should be housed away from noisy areas and machinery that emit loud noise and low frequency vibrations. Sudden loud noises should be avoided. Most birds hear sounds between 1 kHz to 5 kHz with a high frequency hearing limit of 10 kHz for passerines and 7,5 kHz for non-passerines.

C.12 Health

C.12.1 For research purposes, captive-bred birds of suitable health status should be used wherever possible since wild birds can present special or unexpected problems relating to their health and behaviour when held in captivity. Periods of quarantine (normally 30 d) and habituation should be considered before wild birds are used for research purposes.

C.12.2 Health screening and monitoring programmes are recommended. This includes veterinary examinations, faecal sampling, and checks for bacterial and viral diseases and for parasites. Potential zoonoses should be considered.

C.13 Housing and accommodation

C.13.1 A combination of indoor and outdoor housing is encouraged since this is beneficial to birds' physiological and psychological wellbeing. Provision of environmental enrichment and refuge is essential to encourage birds to utilize all available space.

C.13.2 A good standard of health, wellbeing, welfare and scientific research cannot be achieved without satisfactory housing, husbandry and care. Under laboratory conditions, birds spend most of their time in confinement and not undergoing procedures. Standards of husbandry and care in the laboratory should exceed commercial conditions unless the research study has direct justification and application to alleviate a specific problem.

C.13.3 In general, birds should be housed in pens, enclosures or aviaries, rather than in cages. Domestic fowl prefer group housing in larger enclosures.

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C.14 Feeding

C.14.1 General

C.14.1.1 Feeding patterns of wild birds vary considerably, and the nature of the appropriate food, how it is presented, any supplementation, and times it is made available should be considered. The bird's diet should meet the nutritional requirements of the species, and should promote natural foraging behaviour.

C.14.1.2 Dietary enrichment, such as fruit and vegetables, may be given where appropriate. Any changes to the diet (for example, the introduction of commercial feeds) should be done gradually, with retention of a proportion of the original diet to prevent birds going hungry.

Birds have relatively few taste buds but taste is relevant to their diet. Dietary enrichment might have to be considered. Birds learn to avoid unpalatable foods. Diet preferences are shaped by early life experiences, so new foods should be introduced gradually.

C.14.1.3 Some species, such as granivores, require grit to aid food digestion. Grit should be supplied in varying particle sizes since birds will select the suitable size. Grit should be replaced regularly.

C.14.1.4 Dietary calcium and phosphorus might have to be supplied in appropriate form, and at the correct level for each life stage. Shell grit may be fed *ad libitum*.

C.14.1.5 Food should be supplied in troughs rather than circular feeders, which occupy too much floor space, are more difficult to clean and do not allow efficient monitoring of feeding.

NOTE Chicks of some species need to be taught (see C.14.1.6) how to use feeders and water points to avoid starvation and dehydration.

C.14.1.6 Feed for all species should be clearly visible and provided at more than one point to allow equal access for all birds. It is essential to provide sufficient lighting so that chicks can see the food. A few older chicks can assist in teaching the younger ones to eat and drink from troughs and water points.

C.14.2 Watering

C.14.2.1 One watering nipple or cup drinker should be provided for every four to five birds.

C.14.2.2 Care should be taken that chicks cannot become entrapped in drinkers as this can cause them to chill or drown (or both).

C.14.2.3 All birds should have access to water at all times. Water should all be clearly visible, especially for chicks. If juvenile birds do not drink they can be gently "beak dipped" by placing the beak in the water for 1 s to 2 s.

C.15 Substrate, litter, bedding, and nesting material

C.15.1 Suitable substrates for birds should be absorbent, not likely to cause foot damage or lesions, and of particle size that will not cause dust and excessive accumulation on the birds' feet. Where a high incidence of foot lesions occurs, the quality of the substrate should be investigated.

NOTE Suitable substrates are chipped bark, soft wood shavings, chopped straw, washed sand, and sawdust. Sandpaper is not recommended.

C.15.2 Litter should be maintained in a dry, and friable condition. It should be sufficiently deep to dilute and absorb faeces.

C.15.3 To avoid leg splaying and developmental deformities, hatchlings should not be placed on slippery surfaces.

C.16 Environmental enrichment

C.16.1 A stimulating environment is a very important contributor to good avian health. Birds will suffer if they are prevented from carrying out studies that they are strongly motivated to perform. Merely providing adequate space is not sufficient to meet all of their needs.

C.16.2 Perches, dustbaths and waterbaths, nesting sites and nesting material, foraging substrates, simulated natural environment, refuge, pecking objects, and flight and exercise space should all be provided.

C.17 Identification

C.17.1 Non-invasive or minimally invasive methods should be used wherever possible. These include noting physical characteristics and differences, photography, identikit drawings, ringing (closed or split rings), staining or dyeing feathers, microchips, and wing-tagging.

When chicks are ringed, regular monitoring is necessary to ensure that the rings do not restrict growth as the birds develop.

C.17.2 Electronic tagging should be done subcutaneously, or into the pectoral muscle, and never into the bone.

C.17.3 Use of multicoloured leg bands, or combinations thereof, can affect bird behaviour.

C.17.4 Highly invasive methods, such as toe clipping and web punching will cause suffering, and are not acceptable.

C.18 Record keeping

Records shall be kept of all birds produced for, and used in, scientific studies.

The minimum records required are:

- a) bird identifications;
- b) the number of breeding females and males;
- c) the number of mature stock birds;
- d) the numbers of eggs produced or collected;
- e) the number of eggs incubated;
- f) the number of eggs hatched;
- g) the number of chicks reared in brooders;
- h) the nature of research that eggs or birds are allocated;

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- i) health and welfare records, including veterinary and laboratory reports;
- j) deaths, postmortems and findings; and
- k) cage monitoring records.

C.19 Potential welfare issues

C.19.1 Many of the potential welfare problems in birds held in captivity are associated with pecking behaviour such as

- a) aggressive pecking (large groups, insufficient space, grid flooring and bright light);
- b) feather pecking (either of other birds or of their own); and
- c) skin pecking, which can cause serious wounds, suffering and mortalities.

The presence of a few feather-pecked birds can lead to generalised spread of injurious feather pecking.

C.19.2 The following are methods used to reduce feather pecking:

- a) in rearing chicks, the access to suitable substrate and encouragement of foraging and pecking is essential. All chicks should be housed on solid flooring covered with litter;
- b) provision of alternative pecking substrates, bunches of string and blocks of straw;
- c) provision of visual barriers and refuge;
- d) periodically and temporarily lowering light sources and their intensity; and
- e) use of red light or light sources that emit UV.

Methods that cause pain (beak trimming) or distress (low-light intensity for prolonged periods below 20 lux) are not recommended.

C.20 Routine scientific procedures

C.20.1 Blood sampling

The following procedures should be followed for blood sampling:

- a) a site appropriate for the size of bird, size of needle, and quantity of blood required should be selected;
- b) the bird should be restrained carefully to prevent haematoma formation; and
- c) cardiac puncture should be performed under general anaesthesia. This is a terminal procedure.

C.20.2 Administering of substances

The following procedures should be followed for administering substances:

- a) it should be ensured that the least invasive method is used;

- b) recommended doses should not be exceeded;
- c) feathers should never be plucked from live birds; and
- d) intramuscular injections should be avoided whenever possible. It might be necessary to split the total dose between two sites. The recommended dose should not be exceeded.

C.20.3 Surgical procedures

The following procedure should be followed for surgical procedures:

- a) take heed of the special needs of birds and chicks during anaesthesia, and in the provision of post-operative care;
- b) give all birds post-operative analgesia, and administer the first dose before recovery from anaesthesia;
- c) ensure dehydration does not occur by providing fluid therapy as required;
- d) monitor and maintain optimum body temperature throughout recovery;
- e) do not disturb recovering birds unnecessarily; and
- f) monitor recovering birds frequently and after reintroduction to their group.

C.20.4 Monitoring for adverse effects

The following are important considerations for monitoring for adverse effects:

- a) pain should always be alleviated. However, many species have the ability to conceal pain, therefore it should be assumed that, if the procedure would cause pain in humans, it will do the same in the animals (see 6.3.1.1.1);
- b) it should be ensured that all personnel recognize pain symptoms, evaluate the endpoint criteria, and can take appropriate action;
- c) use of observation or animal welfare score sheets (or both) is recommended; and
- d) abnormal behaviour should be regarded as an inability to cope with stress, the environment and procedures.

C.20.5 Anaesthesia

C.20.5.1 Anaesthesia in birds requires training, expertise and a good understanding of avian anatomy and physiology. Special attention should be paid to the trachea, air sacs, lungs, gaseous exchange mechanisms, ventilation triggers, and pre-anaesthetic starvation.

C.20.5.2 The following are important considerations for anaesthesia:

- a) anaesthesia should not be carried out using a cuffed endotracheal tube;
- b) lungs do not collapse when the coelomic cavity is entered surgically;
- c) pain will stimulate respiration;

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- d) birds are prone to hypoglycaemia and should not be starved before gaseous induction. Small birds should not be deprived of food for longer than 3 h;
- e) regurgitation is seldom a problem and is usually only experienced in certain waterfowl or frugivorous birds; and
- f) starvation can reduce hepatic detoxification of certain anaesthetic agents.

C.20.6 Anaesthetic agents

The following anaesthetic agents may be used.

- a) Isoflurane – 2 % is the agent of choice and is safe. Induction and recovery is rapid.
- b) Ketamine – 20 mg/kg to 50 mg/kg (subcutaneous (sc), intramuscular (im) or intravenous (iv)). Recovery is dose related. It is a good sedative but a poor anaesthetic agent with poor muscle relaxation. There is little respiratory or cardiovascular depression with ketamine.
- c) Ketamine (10 mg/kg to 30 mg/kg, iv) + diazepam (1 mg/kg to 1,5 mg/kg, im) or midazolam (0,2 mg/kg, sc, im) are better combinations than ketamine alone (see C.20.6(b)). Recovery and induction is smooth.
- d) Tiletamine and Zolazepam (Zoletil²) (5mg/kg to 10 mg/kg, im). Provides good immobilization and is safe.
- e) Ketamine – (1,5 to 2 mg/kg, im) + medetomidine (im) has the advantage that it can be reversed with atipamazole.
- f) Propofol – (1,33 to 14 mg/kg, iv) has a very high safety margin and is rapidly metabolized. It produces rapid smooth induction with good muscle relaxation, and has a short duration of 2 min to 7 min.
- g) Halothane is not recommended, while ether and chloroform are unacceptable.

C.20.7 Analgesics

The following analgesics may be used:

- a) Buprenorphine – (0,02 mg/kg, im). Duration of effect on various species is uncertain.
- b) Butorphanol – (2 mg/kg, im).
- c) Carprofen – (5 mg/kg to 10 mg/kg, im, per os).
- d) Ketoprofen – (5 mg/kg to 10 mg/kg, im).
- e) Flunixin meglumine – (1 mg/kg to 10 mg/kg, im).

C.21 Release of birds

The following are important considerations for the release of birds:

- a) the welfare and fate of released or rehabilitated birds should be of prime concern;

² Trade name for combination of Tiletamine and Zolazepam.

- b) the possibility of release, and whether birds have to be killed at the end of the study, should be fully considered in the study planning stage;
- c) birds should not be routinely killed;
- d) all legal, practical and ethical considerations should be considered for release back into the wild; and
- e) a stimulating natural environment should be considered to enable released or re-homed birds to adjust rapidly.

C.22 Euthanasia

The following are important considerations for euthanasia:

- a) the preferred method of killing birds is an overdose of an anaesthetic, using an appropriate agent and route. The most acceptable method is an overdose of sodium pentobarbitone. Death should always be confirmed;
- b) ducks, diving birds and young chicks should not be killed by using carbon dioxide. Carbon dioxide is aversive to birds since some diving birds can hold their breath for extended periods and can slow their heart rates. Special care should be taken with these species to confirm death;
- c) if a physical method has to be used, dislocation of the neck is the most humane method, but should be carried out by competent persons;
- d) maceration is acceptable for embryonic birds; and
- e) chilling and freezing are unacceptable.

C.23 Species requirements

C.23.1 Ducks and geese

C.23.1.1 General and special requirements

C.23.1.1.1 Ducks and geese are primarily adapted for locomotion and feeding in water. Their comfort behaviour is bathing and preening.

C.23.1.1.2 Waterfowl have varying abilities to walk and feed on land. Geese are exclusively herbivorous and adapted for land-based feeding and grazing. Ducks can be herbivorous, omnivorous, or carnivorous and adapted for feeding on land or water (or both) to different degrees.

C.23.1.1.3 For research and housing criteria, it is vital to appreciate the habitat and natural behaviour of each species. Many waterfowl species are highly social and live in large flocks. Most are monogamous to the extent that a pair will remain together for at least one breeding season, and, in some geese, for life.

C.23.1.1.4 Some species of duck and geese migrate between Summer and Winter habitats, and become physiologically prepared for such long flights by building up muscle and laying down fat reserves, even in captivity.

C.23.1.1.5 It is important to minimize disturbance since ducks and geese are generally more nervous than domesticated poultry, especially during moulting when they shed all their flight feathers at once.

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C.23.1.2 Water for bathing and swimming

All waterfowl should have some sort of pond (see table C.2), with a stone or grit base for swimming, to encourage natural behaviour and for good feather maintenance. They should at least be able to immerse their heads and shake water over their bodies. Entrance and exit to the water should be easy (especially for juveniles), and ponds should be able to be drained and cleaned periodically. Many species are nocturnal and might make good use of the ponds during the night.

Table C.2 — Pond size and depths for ducks and geese

1	2	3
Type of waterfowl	Area m ²	Depth cm
Dabbling ducks	0,5	100 to 300
Diving ducks	0,5	100 to 300
Geese	0,5	500
NOTE These pond sizes are for 2 m ² enclosures. Ponds may contribute up to 50 % of the minimum enclosure space.		

C.23.1.3 Social housing (see table 2)

C.23.1.3.1 Waterfowl are highly social and form strong attachments with one another. The recommended minimum group size is four birds, which allows two to be removed with the least disruption. Larger groups, with equal numbers of males and females, are advisable.

C.23.1.3.2 Many species become especially territorial during the breeding season. It might be necessary to reduce the group size and ensure adequate space for birds to escape from one another. Lone males might attempt to forcibly mate with females, which can result in injury, distress or death of the females.

C.23.1.3.3 Waterfowl should be housed on solid flooring of suitable material such as a plastics turf or smooth rubber matting. Rough floor surfaces cause foot abrasions and feather damage. Any litter should be dry, friable and deep enough to absorb faeces. Any grids used should be of soft plastics mesh rather than wire, and at least one third of the floor surface should be solid.

C.23.1.4 Environmental enrichment

C.23.1.4.1 General

A stimulating environment will encourage waterfowl to forage, interact, and use all of the available space for behavioural needs. Natural plant cover, artificial refuge, boxes, straw bales, items to pull at (rope or chains secured to walls), pebbles, stones, shell grit, bricks and other non-toxic items may be added to ponds for diving or dabbling enrichment.

C.23.1.4.2 Foraging opportunities

Most waterfowl spend most of their time foraging and feeding. It is important to scatter some food instead of providing it all in feeders. Where food is also scattered into the water, regular weighing is essential to ensure adequate intake is achieved. Access to grass, turf, greens, and grains, is recommended.

C.23.1.4.3 Nest sites and material

Sufficient nest sites and material should be ensured to prevent competition and aggression.

C.23.1.4.4 Feeding space

Fifteen centimetres of feeder trough length per bird is required to allow simultaneous access to feed. Feeder width and length is important to allow birds to shovel food into their bills without hitting the trough sides.

C.23.1.4.5 Housing areas (see 7.6.2)

NOTE Ideally, waterfowl should have larger enclosures with outside access wherever possible. Geese need a larger proportion of dry land space for walking and grazing than ducks. Swimming exercise (including diving) is more important for ducks.

C.23.1.4.6 Welfare issues

The following are important welfare considerations:

- a) **Injuries** caused by collisions with enclosures when flying. These can occur during capture attempts. Pinioning should not be performed routinely as it is a permanent mutilation which can cause acute and chronic pain, and distress.
- b) **Aspergillosis** is a potentially fatal infection caused by the fungus *Aspergillus*. All waterfowl are susceptible to this infection, especially sea ducks. Contact with mouldy feed, bedding and unsanitary housing conditions should be avoided.
- c) **Eye, nostril, feet and cloacal infections.** Waterfowl should be able to immerse their heads in water to prevent dust clogging their eyes and nostrils. Regular access to water helps prevent cloacal and feet infections.
- d) **Abnormal behaviour.** Providing good quality and adequate space, and appropriate social groupings and nesting facilities will reduce both abnormal and stereotypic behaviour.

C.23.2 The domestic fowl (*Gallus gallus domesticus*)

C.23.2.1 Introduction

The domestic fowl has retained much of the biology and behaviour of the wild fowl from which it has descended.

C.23.2.2 Behaviour

C.23.2.2.1 Domestic fowl are highly social and will form groups with stable hierarchies under suitable conditions. Hens prefer to be with conspecifics, prefer familiar birds to an empty cage and should not be housed in isolation.

C.23.2.2.2 Behaviour that is most important to the species is nesting, foraging, perching, scratching, pecking, dust bathing, and roosting. Comfort behaviour is wing flapping, feather ruffling, leg stretching, and feather grooming. Birds should have enough floor space to perform all these behaviour traits.

C.23.2.2.3 Aggression and feather picking are common behavioural problems. Smaller groups of five to twenty birds show less aggression. Subordinate hens often prefer larger groups as these provide increased escape opportunities.

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C.23.2.2.4 Under confined conditions, the optimum male to female (m:f) ratio is 1:4. Under extensive conditions this ratio can be up to 1:20. Males can be solitary or form groups of three to four. Mixed sex groups should contain fewer males to avoid competition.

C.23.2.3 Housing (see 7.6.2.3)

C.23.2.3.1 General

C.23.2.3.1.1 Ideally, domestic fowls should be housed (see table 3) with access to outdoors with appropriate cover and refuge. Social stress is increased with inadequate space.

C.23.2.3.1.2 Domestic fowl are social and should be housed in groups of five to twenty birds, with fewer males than females in mixed groups at a ratio of 1:5 (m:f).

C.23.2.3.1.3 Domestic fowl housed individually for scientific purposes will require special attention to address behavioural needs. Domestic fowl, such as broilers that are bred for specific purposes (for example, rapid growth rates), might be prone to certain developmental abnormalities such as lameness. Special monitoring is required in such cases.

C.23.2.3.1.4 Standard cages with a height of 40 cm that prevent comfort behaviour, and extension of the head and wings, are not recommended.

The minimum recommended enclosure size for group-housed domestic fowl is:

- a) 1 m² per bird less than 600 gm body weight; and
- b) 2 m² per bird over 600 gm body weight.

C.23.2.3.2 Flooring and floor enclosures

C.23.2.3.2.1 Flooring should be solid since this allows provision of substrate to encourage foraging. Hens often prefer to forage than to eat identical feed that is freely available in containers.

C.23.2.3.2.2 Domestic fowl prefer solid flooring to wire floors. A cage is not an appropriate housing environment for domestic fowl. If used, then the cage should have a solid section with loose substrate provided this covers at least one third of the total surface area. If possible, a nest box and perch should be provided.

C.23.2.3.2.3 Domestic fowl kept on wire flooring or without appropriate substrate for pecking and foraging will excessively manipulate food with the beak and revert to feather pecking. This is a welfare concern (see C.23.1.4.6 (d)).

C.23.2.3.2.4 Suitable materials for dust bathing are sand, sawdust and softwood shavings. It is important to replace these frequently, to remove droppings and to reduce disease risks.

C.23.2.3.3 Perching

C.23.2.3.3.1 Domestic fowl have feet that are anatomically adapted to close around a perch when they roost. In captive environments with limited perching space, fowl will struggle to obtain and keep perching space. The welfare of domestic fowl that cannot perch is compromised. Provision of adequate perching space reduces crowding on the floor, allows subordinates some refuge, and reduces aggressive behaviour. Additional welfare benefits are a feeling of safety, enhanced spatial awareness, increased leg bone strength, and improved foot and plumage condition. The condition "Bumblefoot" is commonly due to poor perch design and placement.

C.23.2.3.3.2 Perches should be 3 cm to 4 cm in diameter, and round with a flattened top.

C.23.2.3.3.3 Perch heights vary with breed, age, size, and housing conditions. They should be fixed at 5 cm to 10 cm, and at 30 cm above the floor. Adjustments may be made on observations of birds' use of perching. All birds should be roosting at night, unless the perches are too high.

C.23.2.3.3.4 Each bird should be allowed 15 cm of perch space at each level.

C.23.2.3.4 Nesting boxes

C.23.2.3.4.1 Pre-laying behaviour starts between 20 min and 120 min before oviposition and is characterized by searching behaviour that leads to selection of a nest site and nest building. Hens are strongly motivated to obtain a suitable nest site and will revert to pacing and stereotypic behaviour if deprived of access to nesting areas.

C.23.2.3.4.2 Sufficient nesting boxes should be provided to avoid competition, and access for subordinate birds should also be provided. Psychological stress from not being able to access a nesting site can lead to egg retention and banded eggs (a good indicator of pre-laying stress).

C.23.2.3.4.3 Laying hens should have access to nesting boxes from at least 16 weeks of age. Nest boxes should contain litter and be enclosed and be large enough to allow the bird to turn around in. Wood shavings, clean straw, and wood wool will allow nest-building expression.

C.23.2.3.5 Feeding

C.23.2.3.5.1 Domestic fowl show diurnal rhythms in feeding behaviour with peaks in feeding usually at the beginning and end of the light period. Additionally, the sight and sounds of other birds feeding triggers feeding behaviour.

C.23.2.3.5.2 Provision of insufficient feeding space for all birds to feed simultaneously is detrimental to subordinate or displaced birds. The resultant competition and stress will lead to abnormal behaviour and aggression.

C.23.2.3.5.3 A minimum of 15 cm feeder trough length per bird is required to allow birds of any strain to feed simultaneously.

C.23.2.3.5.4 Husbandry systems should encourage foraging behaviour. Even where concentrated food is fed *ad libitum*, domestic fowl will spend up to 35 % of the day scratching, pecking and foraging.

C.23.2.3.6 Broilers

C.23.2.3.6.1 Lameness is a highly prevalent and painful condition in broilers grown on commercial programmes. This is due to rapid growth rates.

C.23.2.3.6.2 Growth can be influenced by decreasing the daylight length so that birds have less time to feed. Alternatively, reducing the levels of protein and carbohydrates, or restricting feed quantities, will reduce the incidence. However, feed restriction leads to competition, aggression and feather pecking, especially at feed delivery times.

C.23.3 Quail (*Coturnix coturnix*)

C.23.3.1 General

C.23.3.1.1 Wild quail live in small social groups and devote much time to scratching and foraging. Their preferred habitat is denser vegetation, grasslands, riverine bush, and cereal fields.

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C.23.3.1.2 Design of housing for quail should respect these behaviour patterns and provide substrates for scratching, pecking, foraging, dust bathing, refuge and nesting.

C.23.3.1.3 Quail are used in many areas of biomedical and behavioural research. They are suited to reproduction and embryological studies due to their high egg production capacity and rapid maturation rate.

C.23.3.2 Temperature

C.23.3.2.1 Adults

Breeding quail and mature stock should be maintained within a temperature range of 16 °C to 23 °C. At low temperatures (<15 °C), males become inactive and fertility is adversely affected.

C.23.3.2.2 Brooding and growing stock

The critical temperature for hatched chicks is 35 °C to 37 °C. The temperature should be gradually decreased (approximately 0,5 °C per day) to within the acceptable temperature range of 16 °C to 23 °C at four weeks of age.

C.23.3.3 Relative humidity

Quail can be maintained at a relatively wide range of humidity of 30 % to 80 %.

C.23.3.4 Ventilation

Good ventilation is essential to provide birds with a constant and uniform supply of fresh air, and to extract from the area products of respiration, moisture and gases from bedding and droppings. Ventilation rates depend on stocking densities. Draughts at floor level, particularly in brooders, should be avoided.

C.23.3.5 Lighting

C.23.3.5.1 Lighting depends on the purpose for which the birds are being held.

C.23.3.5.2 Light/dark cycles vary from 14 h/10 h to 8 h/16 h (where there is a requirement to slow down growth rates).

C.23.3.5.3 Lighting should be available for newly hatched chicks for 23 h per day, gradually being decreased to 14 h per day at two weeks.

C.23.3.6 Housing and accommodation (see 7.6.2.4)

C.23.3.6.1 Floor pens

Floor pens provide the birds with greater freedom of movement and opportunity for social interaction with less stereotypic behaviour than in cages (see C.23.3.6.3).

C.23.3.6.2 Indoor aviaries

C.23.3.6.2.1 Preference shall be given to floor systems over cage housing. Improved environments can be provided with inclusion of dustbaths, and artificial brush cover. Aviaries with outdoor access are preferred.

C.23.3.6.2.2 Quail have a characteristic escape response that consists of sudden vertical flight movement. Pen heights shall not be too low to avoid head damage. Covering the roof with soft netting or thatch will help prevent injury. A recommended safe height is 60 cm.

C.23.3.6.3 Cages (see table 4)

Cage systems have some advantages over floor systems. Egg collection is maximized, laying production can be monitored, fighting is minimized and head damage is reduced. The major disadvantages are that space is restricted, stereotypic behaviour is common, and foot problems are encountered. Wire cages should be plastics- or epoxy-coated to reduce foot and head damage.

Recommended minimum length of trough per bird is 4 cm.

C.23.3.7 Breeding systems

C.23.3.7.1 The highest levels of fertility and hatchability are achieved in floor pens, with adequate cover provided, and at lower stocking densities.

C.23.3.7.2 In cage systems, trios (two females + one male) are often established at 34 weeks of age. The establishment of mating groups before sexual maturity (eight weeks) will reduce the incidence of aggression and feather picking. Breeding females housed in cages exhibit pre-laying restlessness.

C.23.3.7.3 Small group sizes are more important than stocking densities in reducing mortality rates. Ratios of one male to two or three females is most commonly used.

C.23.3.7.4 The mating behaviour of the male can be brutal and unrelated to the receptivity of the female. Monitoring during mating is required to prevent injury as a result of repeated mating or feather picking.

C.23.3.7.5 Female only groups can be satisfactorily maintained, but fighting is a serious problem in male groups.

C.23.3.7.6 Fertility and egg hatchability are optimal between 8 weeks to 26 weeks of age, but drop off rapidly after this time. Egg production is variable between strains with an average of 70 eggs to 90 eggs laid per bird per 100 d.

C.23.3.8 Incubation

C.23.3.8.1 Special care should be taken in the collection and handling of quail eggs as they are thin-shelled and break easily.

C.23.3.8.2 Hatchability is affected by length of storage before incubation and by the age of parent stock. Storage of eggs post collection is best at 13 °C to 25 °C, for no longer than 7 d. Extended pre-incubation storage will increase the incidence of abnormalities.

C.23.3.8.3 Quail eggs can be incubated in commercial incubators with wire mesh settings or special setting trays. Incubation period is 15 d to 18 d. Hatching should be carried out in the hatching compartment of combined setter hatchers.

C.23.3.9 Environmental enrichment

C.23.3.9.1 Birds should be provided with refuge cover, especially early in life, to reduce fear. Staff should approach quail slowly and calmly. Chicks should be provided with coloured objects (balls, tubes and cubes). Adult birds may be given pine cones, branches, stones, wood shavings, dust-baths (sand or sawdust), nesting boxes, and hay.

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C.23.3.9.2 Females have a strong preference for ground cover (natural or artificial) especially at egg-laying and incubation periods.

C.23.3.10 Welfare issues

C.23.3.10.1 Aggressive behaviour and damage to female birds by the male can be minimized by careful grouping and monitoring of the birds. Males are far more aggressive towards other unfamiliar males. Introductions of new birds should be properly managed to prevent injuries.

C.23.3.10.2 Head injuries might be sustained by birds flying into the roof of the enclosure or pen.

C.23.3.10.3 The composition and quality of the diet is important to reduce skeletal and cardio-pulmonary problems in old caged birds.

C.23.3.10.4 Cage and pen floors should be designed to reduce foot contact with waste food, faeces and water accumulation. Good monitoring of birds' feet is essential. Indoor confined cage housing is not recommended owing to related animal welfare issues.

C.23.3.10.5 Battery housing systems are not acceptable for quail and shall be justified if used.

C.23.3.10.6 Welfare problems increase with age in birds held in captivity, especially those held for more than a year.

C.23.4 Pigeons (*Columbiformes*)

C.23.4.1 General

The most commonly used *columbiform* in the laboratory is the domestic pigeon. In their natural habitat, pigeons usually occur in pairs or larger flocks, feeding and roosting together. They will actively defend roosting and nesting spaces.

C.23.4.2 Social housing

C.23.4.2.1 Pigeons can be housed in mixed groups and will lay eggs, but will not incubate them unless nesting boxes are provided.

C.23.4.2.2 Care should be taken in the choice of breed for laboratory use as some strains show abnormal or undesirable behaviour and should be avoided.

C.23.4.3 Aviary housing

C.23.4.3.1 Consideration should be given to providing sufficient quality and quantity space to allow a full range of behaviour expression, including flight wherever possible.

C.23.4.3.2 Laboratory pigeons are often housed singly in cages that do not allow wing extension or environmental enrichment. Birds thus housed will lose substantial muscle tone and suffer detrimental physiological effects. Small confined cages are not recommended (see 7.6.2.5). Access to flight rooms with perches, for regular exercise, should be provided.

C.23.4.4 Modified cages

Cages for scientific, veterinary or metabolic studies are used in laboratories, but monitoring regimes need to be appropriate for use.

C.23.4.5 Capturing birds

If birds need to be handled frequently, nesting areas should be provided to which birds can retreat for capture. Habituation to these boxes or chambers will reduce capture stress. Where possible avoid excessive manual capture shall be avoided.

C.23.4.6 Floor and substrates

Pigeons should not be housed on grid floors since this prevents normal foraging. Solid floors should be cleaned regularly and with increased frequency where bird densities, and degree of confinement, are higher. Smaller cages require daily cleaning, and attention should be given to high faecal contamination areas (under perches or nesting areas).

C.23.4.7 Perches and nest boxes

C.23.4.7.1 Flight areas and aviaries should allow a separate perching area for each bird, and sufficient box perching to allow birds to establish their own territories. This will reduce aggression and facilitate capture.

C.23.4.7.2 Box perches of width 30 cm, length 30 cm and height 15 cm located in blocks against one wall will simulate a natural environment and allow faeces deposition in one main area.

C.23.4.7.3 Each bird should have 30 cm of perching space.

C.23.4.8 Diet and foraging

C.23.4.8.1 Pigeons are primarily seed-eaters, but are omnivorous and will take a large variety of grains, berries, small molluscs and vegetation. Food that contains animal proteins such as commercially available crumbs and meals may be used, supplemented with legumes and cereals. Vegetable protein alone diet does not provide an adequate diet for pigeons.

C.23.4.8.2 Pigeons (especially females) fed *ad libitum* will become obese, and regular weight monitoring is advised. Obesity can be prevented by restricting the intake to 28,5 g per day for the average weight bird, and by including low palatability grains (barley).

C.23.4.8.3 Cages should have covered food, grit and water hoppers.

C.23.4.9 Breeding systems

Housing birds in mixed groups will help prevent aggression during the breeding season. If breeding is not required this can be prevented by withholding nesting sites and materials. Females will still lay eggs, but will not incubate them without a nesting site.

C.23.4.10 Water for bathing

Waterbaths should be provided at least once per week. Pigeons splash considerably when bathing and will soak the surrounding areas. Care should be taken that this activity does not compromise hygiene management or occupational safety.

C.23.4.11 Environmental enrichment

Pigeons should not be housed in barren conditions in the laboratory. They will benefit from larger cages or aviaries supplied with enrichment items such as nesting facilities, perching, foliage, branches, ropes, swings, mirrors, and substrates.

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C.23.4.12 Welfare issues

Injuries might be sustained during capture, aggression or flight. Appropriate preventive measures and corrective action should be taken.

C.23.5 Finches (*Fringillidae*)

C.23.5.1 General

C.23.5.1.1 Finches belong to the bird sub-family *Estrildinae* (Waxbills). They are delicate and require special care in captivity.

C.23.5.1.2 Finches are highly sociable, usually live in flocks ranging from a few dozen to several hundreds, often mixed with other species of small birds. Flocks are highly mobile, range over wide areas in search of food, and quickly desert areas if conditions become unfavourable.

C.23.5.1.3 Finches are usually monogamous, sexually dimorphic and form long-term pair bonds strengthened by bonding behaviour such as mutual preening. The breeding season usually coincides with the first rains, and the availability of ripening grass seeds.

C.23.5.2 Social housing (see 7.6.2.6)

Fitting finches with brightly coloured leg bands can have a significant effect on social and reproductive behaviour.

C.23.5.3 Aviary housing

C.23.5.3.1 General

C.23.5.3.1.1 In captivity, breeding finches should be housed (see table 5) at an equal sex ratio with an excess of nesting sites. Finches are prolific breeders and will breed best when a small number of pairs are housed together in a medium-sized aviary with sufficient or excess breeding sites.

C.23.5.3.1.2 Communal housing is the best way to house finches, ensuring that plenty of perching space is always available. As a guideline, 20 birds can be housed in an aviary of width 2 m, length 3 m and height 2 m. If space is limited, width can be compromised, but the vertical height and length maintained to allow free flight.

C.23.5.3.1.3 Outdoor access is beneficial wherever possible. Outdoor housing should provide adequate shelter and heating where necessary.

C.23.5.3.2 Solid floor with substrate

Finches feed regularly on the ground. Solid flooring is required. Suitable substrates include bark chips, wood shavings, or sand.

C.23.5.3.3 Perches

C.23.5.3.3.1 Perching provides security and exercise. Perches should be attached at one end so that they are slightly springy. They should be placed at different heights to allow progressive movements up or down. Some perches need to be placed at approximately 15 cm from the roof for night roosting.

C.23.5.3.3.2 Wooden dowel rods or natural branches can be used.

C.23.5.3.3 Too many perches obstruct free flight, and make catching the birds more difficult and increase injury risks. Perches should not be sited over feed and water containers.

C.23.5.4 Diet and foraging opportunities

Diet for finches consists mainly of dried grass seeds, but captive birds do well on mixed seed diet. A few live insects, mealworms, and *Panicum* millet sprays can be provided in both feeders and on the floor. Fresh greens and soaked or sprouted seeds will encourage birds to breed and should not be supplied unless breeding is required. Dry seeds alone will not stimulate breeding.

C.23.5.5 Nest boxes, sites and materials

Finches will actively and aggressively defend nest boxes if these are provided, therefore an excess of boxes, including wicker or plastics hanging boxes shall be provided. If aggression persists, divide the total number of birds into smaller groups and aviaries.

C.23.5.6 Water for bathing

Finches will make good use of waterbaths and these should be provided at least once per week. Depth of water in the baths should be 0,5 cm to 1 cm deep.

C.23.5.7 Environmental enrichment

There is a wide range of commercial toys, swings, etc., designed for companion birds, which can also be used for finches. Varied heights of perching and waterbaths should be provided.

C.23.5.8 Welfare issues

The following are important welfare considerations:

- a) **Feather picking** is usually caused by lack of nesting material for breeding birds, or by overcrowding. Sufficient nesting materials, such as dry grass, coconut fibre and wood wool, should be supplied in hanging baskets made out of chicken wire. These should not be over-supplied as birds will overfill their nests. It might be necessary to remove persistent feather pickers from the aviary.
- b) **Ecto parasites** can cause problems in aviaries, and birds should be monitored regularly and treated as soon as any infestation is detected.
- c) **Hypothermia** as finches are sensitive to low temperatures and rapid temperature drop. Indoor temperatures should be maintained between 16 °C and 20 °C. Adequate shelter and warmth should be provided for birds housed outdoors.
- d) **Coloured leg rings** should be avoided since they affect social and reproductive behaviour. Neutral colours and patterns have far less effect on the birds.

C.24 References

See bibliography.

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Annex D

(informative)

Care and management of laboratory animals — Cattle

D.1 General

Cattle have been domesticated for several thousand years and man has developed a profound understanding of their needs. These needs relate to their physiological and behavioural requirements such as grazing, exercise and them being distinct herd animals. It is important to cater for those needs in order to provide adequate housing and care to cattle.

D.2 The environment

D.2.1 General (outdoors)

D.2.1.1 Cattle can be acclimatized to adverse climatic conditions. For reasons of providing standardized research environments, these animals are often stabled in environmentally-controlled facilities.

D.2.1.2 If cattle are housed outdoors, they require proper shelter from the sun, wind, rain and other adverse weather conditions. They also require access to a dry, well-drained area for rest and rumination. This area should be large enough to accommodate all cattle lying down at the same time.

D.2.2 Temperature (indoors)

D.2.2.1 Cattle housed indoors should generally be maintained at room temperatures between 16 °C and 22 °C.

D.2.2.2 In special cases, for example, when housing very young or recovering animals, higher room temperatures than those indicated (see D.2.2.1) might be required. Gradual acclimatization should be done before moving them outdoors after they have adapted to indoor conditions.

D.2.2.3 Room temperature should be monitored daily, preferably by continuous recording. A less costly alternative is the use of a maximum and minimum thermometer that is examined and reset daily. However, since this does not indicate how long the room was held at a particular temperature, knowledge of which is extremely important, the use of a thermograph is therefore recommended. The temperature of the microenvironment should also be monitored.

D.2.2.4 Occasionally, optimal temperature for the laboratory animal is not the most comfortable for personnel. However, human preferences should not compromise the study requirements or the health and comfort of the animal.

D.2.3 Relative humidity

Humidity control is an important consideration for laboratory animals since it contributes to the variability of research models. For cattle, a relative humidity in the range of 55 % ± 15 % is acceptable. Most animals prefer a relative humidity of approximately 60 %, but can tolerate a range of 40 % to 70 % as long as it remains relatively constant and the temperature range is appropriate.

D.2.4 Ventilation

D.2.4.1 Ventilation influences temperature, humidity, and gaseous and particulate contaminants in the animal cage and holding room. The design of the building ventilation system should permit the maintenance of these parameters within acceptable limits.

D.2.4.2 The actual ventilation rate required varies with age, sex, species, stocking density, frequency of cleaning, quality of incoming air, ambient temperature and humidity, and the type of construction of primary and secondary enclosures, among other factors.

D.2.4.3 Draft-free air exchanges in the range of 10 exchanges to 15 exchanges per hour are commonly recommended for rooms that contain livestock under conventional housing conditions.

D.2.4.4 Differential pressures can be used to inhibit the passage of pathogenic material between rooms. Higher pressures are used in clean areas, as opposed to dirty or biohazardous ones, in order to minimize contamination. Generally, a differential pressure of 2,5 mm to 5,0 mm mercury is maintained.

D.2.5 Lighting

D.2.5.1 The three characteristics of light that can influence laboratory animals are intensity, quality and photoperiod. The lighting should provide good visibility and uniform, glare-free illumination. Light tubes, which imitate the spectrum of sunlight, are commercially available and their use is recommended.

D.2.5.2 Where natural lighting is not used, light and dark periods should be at least 6 h each per day.

D.2.5.3 Photoperiod is probably the most influential of light characteristics on laboratory animals. It is suggested that if a change occurs in an animal's photoperiod, then no experiments should be conducted with that animal for at least a week. If a longer light phase is interrupted by a shorter dark phase, there are few significant effects. However, if the reverse occurs, endogenous rhythms can be significantly skewed. This is one reason why automatic timers should control light cycles in all animal rooms. Timer function should be monitored or hooked into an alarm system. A daily cycle of 12 h dark:12 h light is usual. Additionally, any windows in an animal room should be capable of being blacked out.

D.2.6 Noise

Sudden irregular noises create more disturbances in cattle than continuous or predictable sounds. Noise cannot be eliminated from an animal unit but care should be taken to minimize the generation of sudden extraneous audible and ultrasound noise in the vicinity of animals.

D.2.7 Vibration

Vibration stability is important for the maintenance of a constant study environment for sensitive animals. Therefore, animal holding and test rooms should be located away from areas such as a cagewash, major circulation corridors where racks are frequently in transit, mechanical rooms, and elevator shafts. Vibration studies should be performed to determine how best to achieve the maximum allowable vibration levels as determined by instruments and animals to be used in the area.

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D.3 Animal care and health

D.3.1 General

D.3.1.1 Unless there is good husbandry, veterinary or scientific justification for individual housing, animals should be maintained in compatible sociable groups. These groups should remain stable. Cattle are herd animals which depend on social contact and will show severe stress reactions if separated from their herd. If individual housing is required, the animals should at least have visible contact with conspecifics.

D.3.1.2 Cattle readily establish cohesive social structures. In so doing, they establish hierarchies and inter-individual relationships. When housed in barn-type accommodation, there should be sufficient feeding space, water points and resting areas to avoid confrontations. Where space to avoid conflict is not available (as in most indoor housing), visual barriers should be provided.

D.3.1.3 Cattle respond well to positive food reinforcement such as the provision of barley or lucerne. Low stress handling can be achieved by competent, calm and confident personnel within an environment that is designed to assist such efforts.

D.3.2 Bedding material

D.3.2.1 With the exception of slatted floors, absorbent bedding material such as straw or wood shavings should be added to interior pens to provide a clean, comfortable and dry surface, unless approved otherwise by the AEC for specific study-related requirements. A minimum average layer thickness of at least 10 cm of bedding material is recommended.

D.3.2.2 Bedding may be non-nutritive, but should be non-toxic, absorbent and comfortable. Resinous wood shavings, especially cedar, are not suitable for use as laboratory animal bedding. Pine shavings should be avoided for the same reason, although they are not as toxic as cedar.

D.3.2.3 Slatted floors or cages with grates or perforated bottoms require special caution. Care should be taken that the floors are specifically designed for the breed and weight class concerned, should prevent injuries, provide secure footing, and be comfortable.

D.3.3 Food and water

D.3.3.1 Potable water should be supplied to animals in sufficient quantity and be presented in a manner that an animal can use. Water receptacles should be sited to avoid fouling, while still being accessible to young calves. Tap water might be sufficient for conventional housing facilities. Housing personnel should be aware that adult cattle can consume as much as 45 L per day and up to 90 L per day in hot conditions. It is important to consider that cattle have the ability to consume large volumes in a short time (for example, 20 L in a few seconds). Thus, trough volumes and refill times of bowls, inlet calibre and water pressure are important to guarantee adequate water supply in order to avoid frustration in the animal and possible damage to the trough or bowl. Housing personnel should also ensure that the height of the bunk- or trough-type feeder is suitable for the animals housed.

D.3.3.2 Where large numbers of breeding or stock animals are maintained in pens, it is important to ensure that there are sufficient feeding and watering stations to avoid undue competition. Restricted feeding of groups of cattle is not recommended as it leads to competition.

Consequently, it is recommended that the crib space for cattle is at least 30 cm to 65 cm per animal housed in a pen at a time, with provisions for larger cribs for breeds such as Brahman and Brahman crosses (see also table 6). As for water troughs or bowls, it is more important to provide water

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sources that are adequately placed and provide sufficient volume rather than meet minimum space requirements since dominant individuals can effectively exclude other animals from a water source.

D.3.3.3 Particularly when cattle are allowed to graze on pastures, animal attendants and veterinary personnel should be aware that cattle might ingest material other than normal feedstuffs.

D.3.3.4 An individual animal's nutrient requirements are affected by many factors. Young animals generally need increased amounts of many nutrients. Reproduction places many demands on female animals, and nutrient requirements are very high in gestating and lactating animals. Environmental temperature and humidity can also affect food intake and nutrient needs.

D.3.3.5 All feed should be clean, free of contaminants or pests, palatable, fresh and sufficient for the animal's needs. The selected food should be a balanced diet that provides all required nutrients.

D.3.3.6 The technique of Body Condition Scoring (BCS) should be learned by all herd attendants to assess whether or not the diet of the herd in their care is maintaining the animals in good body condition.

D.3.4 Cleaning

D.3.4.1 Routine cleaning and maintenance, and a high standard of hygiene are essential for good husbandry. Suitable and institutionally approved cleaning agents and procedures should be applied.

D.3.4.2 The facilities should be designed to support manure removal, cleaning and disinfection.

D.3.4.3 Decisions on the frequency of cleaning should be based on the housing system, type of animal, stocking densities, and the ability of ventilation systems to maintain suitable air quality.

D.3.4.4 Fly, tick and other pest populations should be regularly monitored and appropriate control measures shall be applied when indicated.

D.3.5 Environmental enrichment

D.3.5.1 Few environmental enrichment strategies have been published for cattle. It is safe to assume, however, that cattle will respond to changes in feed, as well as to encouragement to display grazing behaviour. The application of the usual principles for environmental enrichment is strongly recommended.

D.3.5.2 The use of positive reinforcement in cattle, with food items such as lucerne, salt or barley as reward, is strongly encouraged.

D.3.6 Animal accommodation (see 7.6.3)

D.3.6.1 Cattle housing facilities should provide suitable access and restraining devices to allow animals to be inspected, caught or moved as necessary.

D.3.6.2 Under natural conditions, cattle spend long periods foraging (grazing) while moving considerable distances. The housing management should take cognizance of this fact and provide access to an outside exercise area whenever possible. Such outside areas should provide sufficient shade and water to accommodate the needs of all animals present at the time.

D.3.6.3 If cattle are maintained over long periods, hoof trimming should be part of the herd management programme.

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D.3.6.4 Pens should be of sturdy construction to contain the animals securely and should be designed and maintained to prevent cattle from becoming trapped or injuring themselves. This is of particular importance in the case of horned animals.

D.3.6.5 Space allowances for cattle vary greatly depending on animal size, breed, presence or absence of horns, gestation status, lactation status, climate conditions, etc. Breeds such as Brahman and Brahman crosses have larger space requirements. In general, pens should be large enough to allow all cattle to lie comfortably on a dry and bedded area. During transport or when in other pens where cattle are kept for short periods, enough space should be allowed for all animals to stand comfortably.

D.3.6.6 Housing cattle in tie-stalls should be avoided whenever possible. Cattle have a particular "swing" technique for getting up and thus require considerable space in front of them. Tie-stalls often restrict this movement. It is also for this "swing" technique that pen sizes are generally more generous for cattle than for other species of similar size.

D.3.6.7 For specific purposes (for example, immediate post-operative care or metabolic studies) it might be justified to restrict the available space or other aspects of the primary enclosure (or both). Such studies should state these conditions clearly in the proposal to the AEC for it to be approved.

D.3.7 Breeding

D.3.7.1 Calving cows should be familiar with their environment and their handlers, and should be allowed to give birth with minimum interference. Animal attendants should be familiar with normal birth and should be able to recognize problems. Assistance in calving should, if necessary, be provided under veterinary supervision.

D.3.7.2 Newborn calves require adequate nutrition and a high level of hygiene. Mothers and their offspring should be disturbed as little as possible.

D.3.7.3 Where slatted floors are used at calving time, cows should be provided with separate pens that have solid, non-slip floors and contain appropriate bedding.

D.3.7.4 Detailed records should be kept of pedigrees as well as of fertility and rearing success.

D.3.8 Animal identification

D.3.8.1 General

D.3.8.1.1 The most important considerations in choosing a marking technique concern its effect on the behaviour, physiology and survival of the animal. Any technique that causes an adverse effect on the animal is not only inhumane, but is likely to distort the data being collected, resulting in meaningless and misleading results.

D.3.8.1.2 In choosing an acceptable marking technique, the researcher should consider the nature and duration of restraint, the amount of tissue removed or damaged, whether or not pain, if inflicted, is momentary or prolonged, and whether the risk of infection and abscessation is minimal.

D.3.8.2 Permanent marking

D.3.8.2.1 Ear-notching is not recommended for cattle.

D.3.8.2.2 Microchips are widely used to uniquely identify animals. New generation microchips even allow for the measuring of body temperature or the storage of animal data on the chip.

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D.3.8.2.3 Ear-tags of a suitable size for livestock are widely available and often used. More than two tags per ear is considered excessive. When reapplying tags, the operator should use the pre-existing hole(s) in the ear.

D.3.8.2.4 Tattoos on one or both ears may also be used. Tattooing should be carried out by an experienced operator, using properly maintained equipment and good hygienic practice.

NOTE Owing to their ease of identification and application, ear-tags have largely replaced tattoos.

D.3.8.2.5 Hot-iron or freeze-branding might be required under some circumstances. If these methods of identification are required, adequate anaesthesia or sedation and analgesia should be provided.

D.3.8.3 Semi-permanent marking

A patch of hair or patterns may be shaved, clipped or cut with a pair of scissors. Such marks generally last from one week to four weeks (depending on the stage of the hair cycle) and can be used on any colour cattle.

D.3.8.4 Temporary marking

Cattle are often marked with marking sticks that leave a strip of colour on the coat. This is easily applied but only lasts for several days, and then it can be reapplied.

D.3.9 Handling

D.3.9.1 Like most animals in research facilities, cattle respond best to gentle and firm handling. Persons working with cattle should avoid sudden movements or actions that might frighten the animals, and should always be alert and observant towards the behaviour displayed by the cattle. This will assist in identifying, for example, aggressive behaviour, so that prompt and appropriate remedial action can be taken.

D.3.9.2 Cattle develop strong habits in their daily routine. This relates to resting positions and the order in which herd members enter the barn. Animal attendants should be aware of such routines and should use them to advantage whenever possible. An example is the placement of a weighing scale or a spray race in the path normally followed by the animals.

D.3.9.3 Cattle are reluctant to enter dark areas and are particularly affected by contrasts between light and dark areas; even the presence of a floor drain can be seen as a contrast. It is thus recommended to ensure good, shadow-free illumination and the absence of any structures that can be seen as an obstacle by cattle.

D.3.9.4 Cattle are also reluctant to move towards sources of noise and unusual smells. Furthermore, they feel trapped and will balk if they see a dead end; they should be able to see a pathway of escape ahead.

D.3.9.5 Apart from calves, which can be restrained manually, most cattle are restrained with the aid of devices such as halters, nose rings, nose clamps, neck clamps or handling chutes. It is of the utmost importance that any such devices are in good working order and adequate for the particular animal and the intended use.

D.3.9.6 Calves should be lifted with proper support for the chest and abdomen and should not be lifted by the head, ears, tail, legs or skin fold.

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D.3.10 Records

Regular monitoring of health and reproductive data, and keeping detailed records thereof, is essential to ensure that problems are identified at an early stage so that corrective action can be implemented to minimize any potentially adverse welfare effects on the animals. This form of monitoring and assessment is of particular importance in herds, where large numbers of animals are maintained, or where there is a high animal turnover.

D.4 References

See bibliography.

Annex E

(informative)

Care and management of laboratory animals — Cephalopods

E.1 General

E.1.1 Cephalopods are large and active molluscs with complicated behavioural expressions. They live in a variety of diverse marine habitats, and behavioural patterns in hunting, feeding, sexual display, attention, sensory discrimination, visual stimuli, conflict and concealment need to be understood. Research on cephalopods generally focuses on basic properties of nerve function.

E.1.2 Researchers intending to work with cephalopods have the responsibility to investigate the anatomy and physiology of these animals in order to provide the correct housing environment, handling techniques, care and maintenance. Cephalopods can exhibit different behavioural patterns in captivity than in deep offshore waters.

E.1.3 Specimens required for laboratory studies generally range from 0,25 kg to 2,5 kg. They require substantial space and provision of waterflow. Most are active, swim by propulsion, and have great mobility. Squid usually hover mid-water using lateral fins. Octopuses are usually bottom or close to bottom dwellers, are very exploratory and are prone to attempt to escape from tanks.

E.1.4 Cephalopods are voracious predators. The octopus, by reason of their natural use of holes and crevices, are the most adaptable cephalopods for captive laboratory use. However, the strength and speed with which the arms and suckers can be used make the octopus a powerful predator and difficult to handle and catch. Squid have an active lifestyle and their rapid swimming habits require substantial space provision. This poses difficulties in keeping squid alive and healthy in the laboratory.

E.1.5 All cephalopods, with few exceptions, will actively catch and eat live prey, and a large range of prey animals have been recorded. Hunting techniques are largely based on visual perception of target prey. Molluscs, worms, fish, prawns, shrimp, and other aquatic groups are natural prey.

E.1.6 Digestive excretion occurs by the release of pigmented material from the digestive gland into the lumen of the gut. Urine drains into the mantle cavity and is then released.

E.1.7 Respiratory exchange with the environment occurs through well vascularised gills suspended in the mantle cavity. Routine oxygen consumption ranges from 10 mL to 500 mL oxygen/kg/h in the squid to 10 mL to 100 mL oxygen/kg/h in the octopus.

E.1.8 Metabolic rates can be increased two to three times by rapid swimming or other violent movements, in addition to the energy demands of digestion processes.

E.1.9 Cephalopod growth rates in captivity and in the field are high. The octopus can show a daily weight increase of 4 % to 6 %, and squid 2 % to 4 % per day. This is due to the high conversion of food intake to growth. The diet is high in proteins, and low in carbohydrates and lipids. Due to their active lifestyle, squid have higher energy demands.

E.1.10 Lifespan is generally short with the average being 1 year to 2 years for most species.

E.1.11 The skin of cephalopods, particularly of the suckers and lips, is liberally supplied with receptor cells responsive to tactile and chemical stimuli. The octopus is very sensitive to light touch

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on almost all parts of the skin surface. Visual acuity is high due to large camera-type eyes situated laterally and dorsally. Octopuses are colour-blind.

E.1.12 Typically all coleoids have an ink sac (muscular bladder) located ventrally and will discharge ink during flight and danger. The ink does not disperse in the water but forms a discrete dark mass.

E.1.13 Sexes are separate and fertilization is achieved by direct mating. After reproduction both males and females die. The consequences of reproduction can have a direct effect on survival and lifespan in captivity.

E.2 Research

E.2.1 The most common reason for holding cephalopods in captivity is for scientific research mostly into aspects of their biology (physiology and biochemistry) and requires supplies of healthy wild-caught animals. They generally need not be held for long periods in the aquarium.

E.2.2 Laboratory research into processes such as growth, reproduction, feeding and metabolism will require animals to be held for extended periods.

E.2.3 Some 45 different species of cephalopods have been successfully maintained in open sea-water circulation systems. There are a further eight species which may be kept in a closed recirculation system under laboratory conditions. The most common species held are the octopods and sepioids (cuttlefish). These adapt to laboratory conditions, provided water quality is adequately maintained and that there is a suitable substrate and supply of appropriate prey.

E.2.4 Squid are adapted to live in open water and tend to dash into the sides of the tank. Damage to the skin surface is the prime cause of premature deaths. It is therefore advisable that suitable tanks be used (see E.6.2).

E.3 Capture methods and sources

E.3.1 Most cephalopods have a soft delicate skin surface that is easily damaged by mechanical abrasion, bruising, striking rough objects, skin stretching and rough handling. Poor capture techniques of juveniles and adults often results in high mortalities.

E.3.2 Cephalopods are usually sourced from the fishing industry as "secondary" or "by-catch". The condition of these animals shall be assessed before being transported to laboratories.

E.3.3 For octopods, the use of traps or pots is preferred as the octopods will take up residence in these for shelter. Pots should be lifted carefully every 2 d to 3 d. Hand collection by scuba diving is also used in certain locations.

E.3.4 The use of bottom and pelagic trawls, seine nets, gill nets, lift nets and jigging lures is considered unacceptable.

E.3.5 The eggs of octopods and sepioids are laid in protective capsules and attached to hard surfaces on the bottom of the sea, or on buoys or ropes. Hand collection of eggs can provide suitable laboratory material. Alternatively, mature adults brought into the laboratory could provide a source of eggs.

E.4 Handling and transport

E.4.1 Every effort should be made to avoid skin damage and bruising. The skin is easily damaged by dry surfaces. Cephalopods of all types should be kept moist and should not be exposed to the air for extended periods.

E.4.2 Small octopuses and sepioids may be placed in temporary containers partly filled with seawater which should be changed regularly if the temperature, pH value and oxygen content alter significantly.

E.4.3 For transport times of longer than 2 h, provision should be made for cool boxes and extra water. Larger specimens may be contained in a polythene bag, filled one third with seawater and the remaining space filled with air (preferably oxygen). The bags should be sealed and kept cool.

NOTE Survival of 8 h to 10 h is possible with this method. Small octopuses, hatchlings or egg masses can be transported by these methods over long distances.

E.4.4 Animals captured at sea are best held in deck tanks of seawater, continuously pumped fresh from the open ocean. Pumping of harbour water is unacceptable because of high contamination levels. Squid will require larger tank space than octopuses.

E.5 Water quality

E.5.1 Two basic types of seawater aquaria are used. Marine or coastal laboratories can use an open system. Inland laboratories generally use a closed or a recirculation system in which a fixed volume of water is pumped from a reservoir, through holding tanks with degrees of conditioning. A continual replacement of a portion of the water volume is recommended.

E.5.2 The main parameters for the monitoring of water quality are temperature, salinity, pH, oxygen concentration, and levels of dissolved nitrogen (ammonia (NH₃), nitrite (NO₂), and nitrate (NO₃)). Water temperature should be as close to ambient as possible.

E.5.3 Cephalopods are stenohaline (live in a narrow salinity range). The optimum is as close to full strength seawater as possible. They are also sensitive to acidity, and the pH value level shall be held above a pH value of 7,5. Normal seawater pH value range is 7,8 to 8,0. Low pH value levels can be corrected by the addition of sodium bicarbonate. Dissolved oxygen levels should be maintained close to saturation levels by forced aeration.

E.5.4 The build up of nitrogenous excretory waste products is a major problem for closed seawater aquaria. Acceptable standards are less than 10 mg/L ammonia, 10 mg/L nitrite, and 20 mg/L nitrate.

NOTE Some octopuses might tolerate a slightly higher nitrate level.

E.6 Space requirements

E.6.1 Certain sedentary species can be held in small enclosures, provided there is an adequate rate of exchange of water to maintain quality.

E.6.2 Octopods and sepioids quickly establish themselves in tanks of modest dimensions. Circular tanks are the best design.

E.6.3 Factors such as aggression and activity will determine tank size and numbers held.

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E.7 Housing and substrate

E.7.1 Squid require no special substrate or housing, but low-light intensities and shielding from external disturbance are essential. Seawater drains should be covered with netting to prevent animals becoming trapped. Plastics sheeting or netting should be used to cover the tank tops to prevent animals getting out, and to reduce visual disturbance from external movement. Optimal lux levels are 10 lux to 15 lux at the middle of the water column.

E.7.2 Glass or plastics viewing panels may be placed in the tank sides. Movable black plastics curtains may be used to confine squid to smaller portions of the tank for capture and cleaning purposes. Capture may be done gently using a lift net.

E.7.3 Cuttlefish and other sepioids will generally make use of any sediment or substrate of the right grade on the tank floor. They will partially cover themselves with sand and thrive well if this is provided.

E.7.4 Octopuses require plenty of shelter in the form of earthen flowerpots, polyvinyl chloride (PVC) pipes, and small PVC kennels. Provision of sufficient adequate shelter will allow a higher stocking density. Tank lids should be close-fitting and secured since octopuses might push loose lids off and escape.

E.7.5 Any uneaten food should be removed as soon as possible from the tank.

E.8 Health hazards

Normal feeding methods of octopuses can pose a health hazard to human handlers. Many species are known to bite with beaks and can cause appreciable wounds. Their saliva contains a wide variety of pharmacologically active compounds that have toxicological and painful effects in vertebrates. When handling the octopus, care should be taken not to allow the mouth area to come into contact with the handler's skin or for the animal to crawl freely over the handler's skin. Octopuses should be handled firmly but with confidence.

NOTE Potentially pathogenic organisms as well as health risk nematodes, such as *Ascaris* and *Anasakis*, have been cultured from the oral regions of octopuses.

E.9 Feeding and food supplies

E.9.1 Feeding cephalopods are almost exclusively predatory carnivores and require a supply of live food. No artificial diet is currently available.

E.9.2 Octopuses capture and eat almost any crustacean of appropriate size, crabs, shrimps, prawns, squat lobsters, some varieties of gastropods and molluscs.

E.9.3 Squid and cuttlefish catch fish and pelagic crustaceans. Food provided should be from a supply of choice of prey and size range encountered in the normal habitat of the species. Suitable prey size should ideally not exceed 10 % of the mass of the cephalopod predator.

E.9.4 Octopuses can survive without food for periods of several weeks but feeding rates of healthy growing cephalopods are high. For cool temperate, warm water and tropical species food of 1 % to 10 % of body weight are required for octopods and up to 15 % of body weight for squid. Flesh retrieval from crabs, for example, is approximately 50 % and the retrieval rate should be taken into account for the actual available food mass. Food should be supplied *ad libitum*. Octopus will attack and eat dead sardine if this is presented on a skewer and moved around.

E.10 Growth

E.10.1 High food intakes, coupled with exceptional growth rates and feed conversion efficiency, results in high growth rates. For octopuses, 100 g of crabmeat ingested results in a 40 g body mass increase and this can be used as a guideline.

E.10.2 Ratios for growth increment to food intake ranges from 40 % to 60 % for octopods and between 25 % and 40 % for squid.

E.10.3 During the juvenile phase, growth rates (body mass increase) per day for octopods is 4 % to 8 %, and for those nearing adulthood and adults this decreases to 1 % to 2 %.

E.11 Lifespan

E.11.1 The second and slower phase of cephalopod growth marks the onset of sexual maturation and the beginning of the final phase. Physiological changes associated with the final stages of gonad formation and vitellogenesis does not seem reversible.

E.11.2 After spawning, females die, almost without exception, within a couple of weeks. Males usually die after the first breeding season.

E.11.3 Larger species of octopods can live from three years to five years in the natural habitat but only one year under laboratory conditions.

E.11.4 Most cephalopods which reach full maturity in the laboratory will not spawn normally.

E.12 Damage and diseases

E.12.1 Wild-caught cephalopods are prone to mechanical damage. Cuts, abrasions and bruising are the most commonly encountered due to rough handling or capture methods and equipment. Octopuses are hardier than squid.

E.12.2 Internal damage to muscles and bruising shows as conspicuous swellings which are coloured blue due to blood leakage and accumulation. Nerve damage is seen as paralysis of one or more arms, asymmetrical stance, head not held level or permanent white skin due to chromatophore damage. Infected lesions invariably result in death.

E.12.3 Long-term laboratory held octopuses might develop ulcerations on the skin. These will spread rapidly in the epidermis and soon affect the dermis and underlying musculature. Skin bacterial and fungal infections are often serious complications and can be related to high-density stocking rates and intensive rearing programmes.

E.13 Parasitism

Cephalopods carry a wide variety of parasites and symbionts, including viruses, bacteria, fungi, sporozoans, ciliates, cestodes, nematodes, polychaetes, copepods, and isopods. Few of the parasitic organisms cause problems in the laboratory. *Ascaris* and *Anasakis* are potential health hazards to humans.

E.14 Cannibalism

E.14.1 Where cephalopods are held collectively or at high stocking densities, cannibalism can occur. Small octopuses are killed and eaten by the larger ones.

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E.14.2 Animals that are sick or are dying are commonly eaten by others even when still alive.

E.14.3 Injured animals, especially those with damaged blood or damage to nerve supplies to an arm, will self-mutilate and eat necrotic tissue. These animals should be put to death by recognized euthanasia methods.

E.15 Culture and breeding

E.15.1 Culture and breeding under laboratory conditions is difficult and success will require specially favourable open seawater conditions or high-quality recirculation systems.

E.15.2 Handling of hatchlings and adequate appropriate food supplies are major concerns in laboratory and aquaria management.

E.16 Maturation

E.16.1 Maturation in females is largely a process of gonad growth and yolk accumulation. The later stages take place under the influence of gonadotrophic hormones from the optic gland. Feeding and growth rates of females in aquaria decline quickly with the onset of maturation. They become relatively sluggish and might deposit eggs on the side of the tank or other hard objects such as pots, stones and pipes.

E.16.2 Fully mature animals can be recognized by the white appearance of the ovary through the muscle wall and changed appearance of the mantle.

E.16.3 The pattern of maturation is similar in the squid, except that the oviduct is single and found on the left side. These animals rarely survive anaesthesia or surgery for internal examination purposes.

E.16.4 Males are ready to mate over a substantial portion of their lifespan. The total mass of ripe testis and full spermatophoric sac does not contribute such a large proportion of the body mass as does the female ovary.

E.17 Sexing

Male and female octopods can be distinguished externally by the presence of modifications of the arm used for sperm transfer in mating by the mature male. In octopods, it is the third arm that becomes thickened. A fold in the skin along the arm develops and the tip of the arm becomes modified and hook-like. Sepioids can usually only be examined and sexed after death, but octopods can be examined and sexed under anaesthesia.

E.18 Mating

E.18.1 Octopuses frequently mate in aquarium conditions. Mature males and mature females mate readily in the same tank. Fertilization is internal and timing is essentially determined by the female. Squid are more reticent and often exhibit chromatophore displays which function as intraspecific visual signals.

E.18.2 Female squid have various methods of holding male sperm in the buccal cavity, within the mantle cavity, or attached to the base of the gills.

E.18.3 Mating in cephalopods is always one-to-one although each individual can perform a series of matings with different partners.

E.19 Egg laying

E.19.1 Many octopod and sepioid species will lay viable eggs in aquarium conditions. This usually occurs when gravid females close to egg-laying are brought into the aquarium. A number of species, especially those that lay large eggs, might lay eggs after long-term rearing, dependent on optimum environmental conditions.

E.19.2 Sepioids lay small numbers (25 to 1000) of large eggs, 1 mm to 10 mm in diameter, within a period of a few weeks. Eggs are individually deposited, firmly attached to a hard substrate, and each enclosed in a tough sheath that increases the size of each egg.

E.19.3 Octopods lay large eggs of length 2 mm to 15 mm and from 25 to 50 and up to 100,000 in number in some species. The eggs are in strings and usually attached in the protection of rocks, pots, and overhangs. Egg-laying can be completed in a day or can take up to several weeks to complete. Females usually brood over the egg masses and protect them from predators. This behaviour continues until hatching of juveniles is completed.

E.19.4 Culturing egg masses requires gentle circulation of clean aerated seawater. Direct agitation of the water from stirrers, skimmers, or aerators shall be avoided and low-light levels shall be maintained.

E.20 Hatchlings

E.20.1 Development time in the egg depends on the species and temperature. It generally ranges between 10 d and 100 d. Hatching embryos actively break out of the enclosing egg coats.

E.20.2 Hatchlings should be reared in separate facilities away from adults and other potential predators. A series of replicated small tanks provide security and easy management. Young growing squid require increased space to swim. These early stage juveniles are vulnerable to excessive water movement and over-aeration.

E.20.3 Inflow and outflow pipes should be screened with fine mesh. Screened aeration inlets should be provided to protect hatchlings from air streams.

E.20.4 For the first week, hatchlings will feed on the remains of the egg yolk and thereafter will begin to feed on live food (appropriate crustaceans).

E.20.5 Hatchlings require high-quality seawater. Artificial seawater will produce a high proportion of defective juveniles (unco-ordinated swimming and walking, corkscrewing and somersaulting). Correction of the trace elements (such as strontium) is essential.

E.21 Handling

E.21.1 Most common trauma is related to rough handling, self-inflicted damage, the animal striking tank walls and rough dry surfaces, failure to keep animals moist, exposure to air (even for short periods), low oxygen levels, high levels of ammonia, and high temperatures.

E.21.2 Handling can produce severe physiological stress which results in changes in plasma levels of glucose, catecholamines, corticosteroids, adrenaline and noradrenaline. The effects of physiological stress shall be taken into account when determining the adaptation period in the laboratory. Cephalopods have complex behaviour, neurological and hormonal control systems that require the utmost attention to the physiological consequences of capture and laboratory management.

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E.21.3 Octopuses in the laboratory may be handled directly or by hand nets. Most handling should take place underwater and contact with dry, rough and absorbent surfaces should be avoided. Animals should be gently coaxed into buckets, tanks or nets, and not forced.

E.22 Identity marking

E.22.1 Identity marking is generally not a recommended practice owing to soft body tissues and delicate skins which are not conducive to the attachment of tags. Octopuses have been identified in the field by using plastics discs attached to both sides of the arm with nickel pins.

NOTE Under laboratory conditions, the use of separate enclosures is the preferred method for identification purposes.

E.22.2 Inert coloured latex implants implanted beneath the ventral mantle skin may be used but should be inserted under anaesthesia, which will require additional handling.

E.23 Anaesthesia

E.23.1 Only anaesthesia by immersion is recommended.

E.23.2 Squid will not normally recover from handling or surgery under an anaesthesia. Animals that are in really good condition may be briefly anaesthetised for weighing or photography.

E.23.3 Octopods can be transferred to a suitable tank that contains seawater and an anaesthetic. Depth of anaesthesia is controlled by the concentration of anaesthetic and the duration of immersion. Signs of anaesthesia are progressive loss of activity and paling of the skin, and ventilatory movements slow down and stop. At this stage anaesthesia is considered complete. Local movements of the arms and skin, and reflex contractions of the mantle, can still occur but there is no co-ordinated or direct activity.

E.23.4 As fully anaesthetised animals have stopped breathing, they will begin to asphyxiate from that point onwards even though the heart and circulation continue to function. A 10 min to 20 min period is considered to be the safe limit after which animals shall be returned to clean aerated seawater. Recovery period is usually 5 min to 6 min.

E.23.5 Ambient temperature seawater should be used.

E.23.6 Substances used for anaesthesia include :

- a) urethane (3 % in seawater); and
- b) ethanol (or industrial methylated ethanol) (2 % to 2,5 % in seawater).

E.23.7 Side effects that might be encountered are attempts to escape from the container and violent inking.

E.24 Surgical techniques

Octopods generally withstand surgical procedures carried out under anaesthesia well, provided procedures are carried out quickly and efficiently with minimal trauma.

E.25 Euthanasia

E.25.1 The simplest and most humane method of killing an octopod is by terminal anaesthesia. Death should be confirmed by destruction of the brain by a scalpel.

NOTE The brain is situated directly between the eyes.

E.25.2 Squid are killed by decapitation, which involves cutting between the head and the mantle.

E.26 References

See bibliography.

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Annex F

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Care and management of laboratory animals — Dogs and cats

F.1 General

F.1.1 The process of domestication has led to dogs and cats becoming convenient animals for laboratory use. Prime areas of use are for teaching, regulatory pharmacological testing (safety and efficacy), feed trials, testing of ecto-parasitocides and endo-parasitocides, vaccine testing and development.

F.1.2 Many dogs and cats used for scientific purposes are purpose-bred. Others are acquired from shelters, pounds and informal settlements, but there are risks involved with obtaining animals from these sources (see 7.2.4). Animals used for scientific and testing purposes are ideally obtained from reputable or registered sources.

F.1.3 The choice of breed should be justified on ethical grounds, considerations for scientific purposes, on what produces the best scientific results, and not based simply on economics. The beagle is the most commonly preferred dog breed because of its relatively small size and placid temperament, its ease of handling and housing, and the volume of scientific and genetic data available on the breed.

F.1.4 Animal housing should always be designed in such a way that the behavioural and welfare needs of the animals are not compromised by the requirements of the scientific research.

F.1.5 Everyone involved with animal use should question any study that restricts the potential to provide good quality environment and care for the animals.

F.1.6 Careful consideration should be given to balancing supply with demand within establishments, and to reduce surplus animals being produced.

F.2 Legislation and responsibilities

All relevant Acts and Regulations relating to the care, housing, and treatment of animals shall be complied with (see clause 1).

F.3 Behaviour

F.3.1 Good husbandry and care depends on a sound understanding of the animals' behavioural needs, the interpretation of animal signals and the perceptual abilities of the confined animals.

F.3.2 Aside from breed differences, there is a wide variation in the temperament of individual dogs and cats and their responses to housing conditions, husbandry practices, and scientific procedures.

F.3.3 Dogs and cats are social animals and should be housed in groups. Management practices should ensure regular contact with other conspecifics, and humans, to promote the welfare of the animals and to encourage the development of properly conditioned animals of suitable temperament.

NOTE There might be instances where individual cats are unable to cope with group housing. Personnel working with cats need to be aware of these possibilities.

F.3.4 Cats are excellent climbers and prefer to spend much of their time on shelves raised off the floor. Pen design should take this strong behavioural instinct into consideration.

F.3.5 Where animals are kept in confined spaces, they are under increased social pressures and the range of defence strategies at their disposal might be limited.

F.3.6 Animal care staff should be trained to recognize the animals' aggressive signals and act accordingly using established husbandry routines.

F.3.7 The need to retain animals showing marked abnormal or stereotypic behaviour should be questioned since this constitutes an ongoing welfare problem and is detrimental to scientific research data.

F.3.8 Cats have a strong territorial nature and are therefore likely to become stressed if relocated.

F.3.9 Cats can develop complex hierarchical relationships. Therefore, a very important management aspect is the consideration of adequate space provision for the number and location of feeding, drinking, sleeping and elimination points in group-housed enclosures.

F.4 Housing and husbandry

F.4.1 Location

F.4.1.1 The location and construction of the animal facility should comply with institutional, local municipal and government regulations.

F.4.1.2 The facility should be situated away from sources of noise, pollution and traffic likely to cause stress or injury to the animals.

Areas that are prone to flooding and poor drainage should be avoided, especially where outdoor kennels and runs are planned.

F.4.1.3 Access to the site area should be controlled and shall be for authorized personnel only.

F.4.2 Construction

F.4.2.1 All facilities should be properly planned and be of sound engineering design. Advice should be sought from a qualified animal behaviour specialist when designing accommodation and exercise areas.

F.4.2.2 Materials used to construct pens and enclosures should be independent of the primary structure, so as to allow refurbishment and improvements with minimal disruption.

F.4.2.3 Kennels, pens and cages should be separated by either solid partitions or mesh wire dividers capable of preventing physical contact with neighbours.

Any wire mesh partitions should be strong enough to contain the animals held, and not cause injury. The recommended mesh size should not exceed 50 mm².

F.4.2.4 Internal surfaces, with which animals have contact, should be constructed of impervious materials to facilitate cleaning, disinfection and drainage. Concrete should be sealed and be smooth. Floors should be non-slip.

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F.5 Pens and cages

F.5.1 Terms

The following terms are commonly used in reference to pens and cages:

- a) **Night box** – kennel with no run, principally for the animal to sleep in.
- b) **Run** – an area allowing space for the animal to exercise and defecate.
- c) **Kennel** – a night box with a run attached.
- d) **Exercise yard** – an area separate from the kennel(s), in which the animal(s) can be released for exercise and socialization, including with humans.

F.5.2 Pen and cage size — Dogs (see tables 7 and 8)

F.5.2.1 Communal housing

F.5.2.1.1 The mixing and introduction of dogs for short-term housing (up to a few weeks) should be discouraged owing to risk of disease transmission.

F.5.2.1.2 Early clinical signs of illness are more difficult to observe in a communal group. In addition, submissive animals are likely to have their food taken by dominant animals.

F.5.2.1.3 If dogs are kenneled in communal groups, unsterilized males should not be housed together. Bitches in season should be kenneled separately.

F.5.2.2 Individual housing

F.5.2.2.1 Social isolation should be avoided.

F.5.2.2.2 Pens which house two compatible animals allows for social contact and facilitates the proper management of kenneled dogs because it ensures that each dog's food and water intake plus health status can be easily observed and assessed.

F.5.2.2.3 Dogs will experience varying degrees of stress by being confined to a pen. Aggressive behaviour (towards each other) can manifest when groups are confined in close proximity. Dogs should therefore have access to exercise runs at least twice daily for enrichment and stimulation, as well as to prevent withered muscles and inactive joints.

F.5.3 Pen and cage size — Cats (see tables 9, 10 and 11)

F.5.3.1 Communal housing

F.5.3.1.1 The mixing and introduction of cats for short-term housing (up to a few weeks) should be discouraged owing to risk of disease transmission.

F.5.3.1.2 Cats should have access to an exercise area twice daily, at least for 1 h, if not adequately provided for in the housing pen.

F.5.3.2 Individual housing

F.5.3.2.1 Social isolation should be avoided.

F.5.3.2.2 No more than two cats from the same source should be housed in any individual housing unit (see 7.6.5.3.2).

F.5.3.2.3 Cats should normally be housed socially (except the periparturient queen) (see 7.6.5.3.3). It is important that there is adequate social contact with other cats, and humans, during the primary socialization period (3 weeks to 14 weeks). Post-weaned cats may be kept in same sex groups.

F.5.3.2.4 The duration of single post-weaned housing for scientific purposes should be kept to an absolute minimum, and be closely supervised.

F.6 Physical environment

F.6.1 Drainage

Kennels and cattery floors should be sloped to allow adequate drainage of waste and water.

F.6.2 Temperature

F.6.2.1 Dogs and cats have wide thermoneutral zones and most species can be held at ambient room temperature without adverse effects. Suitable contingency plans should be prepared to deal with abnormal variations (hot Summer, cold Winter, heating or air-conditioning failures) and to maintain a comfortable environment, especially for very old or very young animals.

F.6.2.2 The optimum temperature range for adult dogs and cats is 15 °C to 23 °C. Heating or cooling will be required if animals are held indoors for prolonged periods outside of this range. Outdoor housing should provide shelter against adverse weather conditions.

F.6.2.3 Newborn pups and kittens require a local environmental temperature range of 26 °C to 28 °C for at least the first 5 d to 10 d of life.

F.6.3 Relative humidity

Dogs and cats will tolerate a wide variation in relative humidity of between 30 % and 70 %.

F.6.4 Ventilation and air changes

Ventilation should be adequate to keep housing free of dampness, noxious odours and draughts. For dogs and cats held at the maximum stocking densities, draft-free air exchanges in the range of 10 exchanges to 15 exchanges per hour are required for all enclosed areas. Lower stocking densities may permit fewer air exchanges.

F.6.5 Lighting and light intensity

F.6.5.1 The photoperiod may be varied in cat colonies to control the reproductive cycle. Normal photoperiod allows 14 h of light but may be reduced to 8 h where reproductive cycle control is desired.

F.6.5.2 For dogs, the photoperiod should not be less than 12 h light. Where natural light is excluded, provision of low-level night lighting can be of benefit to dogs.

F.6.5.3 Lighting should be adequate to allow safe working and cleaning conditions, and efficient inspection of all animals.

F.6.5.4 Natural sunlight is the preferred means of lighting, but shaded areas are essential.

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F.6.6 Noise

F.6.6.1 Cats are sensitive and easily startled by sudden loud noises. Therefore, cats should be housed away from noisy or high activity areas such as delivery, waste disposal and waste collection areas (see 7.5.4.5). They should also be housed away from dogs.

F.6.6.2 Background sounds, such as soft music, are considered beneficial.

F.6.6.3 Noise-producing equipment and machinery should be sited as far away from the animal housing as possible.

F.6.6.4 Kennelling and pens should be designed using suitable sound-absorbing materials to minimize noise levels, but the materials used should not compromise hygiene and cleaning requirements.

F.6.6.5 Elimination of vocal chords of dogs or use of electric shock bark collars are not acceptable.

F.6.7 Bedding

Beds are recommended for sick animals, post-operative recovering animals, older animals (to reduce bed sores), and for periparturient and suckling mothers.

F.6.8 Security

F.6.8.1 Kennels and cattery buildings should be locked and secure when no-one is in attendance. Remote control and swipe cards are recommended for use, with interlocking doors. Catteries should have a double door system to prevent escape.

F.6.8.2 Each individual kennel, cat cage, unit, module or colony should be fitted with a secure closing device that cannot be opened by the animals being held.

F.6.8.3 Where dogs are housed, a security barrier of at least 3 m high should be constructed to prevent animal escape or unauthorized entry.

F.7 Food and feeding

F.7.1 Sufficient palatable food of adequate nutritional value should be supplied daily to each animal. Dogs and cats show a clear preference for a meat-type diet rather than cereals, ground meat rather than cubed meat, and moist rather than dry feed. Providing a variety can prevent monotony in the feeding of long-term housed animals.

F.7.2 New arrivals, and particularly young animals shortly after weaning, should be fed the same diet as at the previous establishment. Any diet changes should be done gradually over 7 d to 10 d.

F.7.3 Where commercial foods are fed, the manufacturer's instructions should be adhered to.

F.7.4 Feeding of all categories of brood bitches and queens, growing pups and kittens, older animals and sick animals should be researched to fulfil all nutritional requirements and special diet regimes (*ad libitum*, restricted, or as per research study criteria).

F.7.5 The condition of the animals as well as the food intake should be monitored. Weight gains and losses should be noted, and causes for concern identified.

F.7.6 Ideally, cats should be offered their daily food requirements, in divided portions, several times per day.

F.7.7 Group-housed cats require one food bowl per cat. The food bowls should be placed in two or three different areas of the enclosure, preferably at different heights. Feeding areas should not be placed near litter trays to prevent contamination of food.

F.7.8 Animals should not be exercised directly after feeding.

F.7.9 For animals with poor appetite, providing a secluded and private place to eat will decrease stress and competition. Warming food, or adding additional enticements, might be necessary.

F.7.10 Spoiled, old, or mouldy food should be removed and disposed of. Residual uneaten food will attract vermin.

F.7.11 Food should be hygienically prepared in a separate diet kitchen area. It should be stored appropriately and in accordance with manufacturer's recommendations. Perishable foods should be refrigerated, and dry foods should be kept in a cool, vermin-free storage area.

F.7.12 All food bins should be covered with lids. Feed bowls should be non-chewable, non-spillable, and be able to be cleaned and disinfected at least daily.

F.7.13 Water should be clean, potable and unrestricted, and should be provided at more than one water point for group-housed animals.

F.8 Health

F.8.1 General

Dogs and cats require frequent human contact, and quality time should be spent with each animal.

F.8.2 Disease prevention

F.8.2.1 Dogs and cats require regular vaccinations and treatments for external and internal parasites. A vaccination and treatment case history should be kept for each animal. Animals that enter the facility should be accompanied by such vaccination and treatment certificates.

F.8.2.2 The consultant veterinarian should provide advice on vaccination, treatment, and quarantine or isolation programmes.

F.8.2.3 Dogs and cats less than four months old, or animals suffering from an infectious disease, should not enter the facility but, in exceptional circumstances, they should be held in isolation.

F.8.3 Health checks and veterinary attention

F.8.3.1 Each animal should be checked at least twice a day to monitor health and comfort. Any changes in health status should be reported immediately to the person-in-charge.

F.8.3.2 A fulltime or consultant veterinarian should be appointed to oversee the health and management requirements of the facility animals.

F.8.3.3 All unexpected deaths should be thoroughly investigated by the veterinarian and any necessary action be taken.

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F.8.4 Quarantine and isolation

F.8.4.1 Appropriate facilities should be available for the quarantine of newly introduced animals, and the isolation of animals suspected of, or which have been diagnosed as, having an infectious condition, and which pose a risk to the health of other animals or humans.

F.8.4.2 Facilities should be designed in such a way as to prevent spread of disease by cross infection. Facilities should be able to be easily cleaned and disinfected. In large establishments, it is recommended that 10 % of the boarding capacity be available for isolation purposes.

F.8.4.3 Animals that have been in contact with an infectious disease case should be isolated from both the infectious case and all healthy animals. Veterinary advice should be sought in the management of specific disease outbreaks.

F.8.5 Medication

F.8.5.1 The person-in-charge and staff should follow all written medication and treatment protocols, unless they receive advice from the veterinarian to change or to terminate the protocols.

F.8.5.2 All medications and treatments should be recorded on the individual animal's record card. The veterinarian's signature, and all instructions, should also be recorded.

F.8.5.3 Where authorized staff administer medications, records should be kept of all administrations and filed for reference purposes.

F.8.5.4 Prophylactic medication should not be a substitute for good hygiene.

F.8.6 Exercise

F.8.6.1 General

F.8.6.1.1 Dogs and cats should have the opportunity to exercise to allow them to:

- a) urinate, defecate and explore the environment;
- b) have contact with other animals of their species (where appropriate) and with humans;
- c) allow muscular activity; and
- d) have their behaviour, mobility and locomotion monitored by staff.

F.8.6.1.2 Dogs and cats spend a lot of their time resting, but this should not lead to an underestimation of their requirements for physical and social interaction during their active periods.

F.8.6.1.3 Exercise provides mental and physical stimulation and should be a daily activity. It should be carried out in a designated and specially designed and equipped area as this increases stimulation and choice of activity. Outdoor exercise facilities, with access to shade, are recommended.

F.8.6.1.4 Animal care staff should be on hand to supervise exercise studies, and to interact with animals.

F.8.6.2 Dogs

F.8.6.2.1 Exercise can be provided by allowing dogs to an exercise area for at least 15 min twice daily, or by walking them on or off the lead for at least 15 min twice daily.

F.8.6.2.2 Very active dogs might require more exercise and older dogs less exercise.

F.8.6.3 Cats

Cats should have enough pen space (see 7.6.5.3) to allow them to stretch and move freely.

F.8.7 Disposal of animals and biological waste

The person-in-charge should have a documented policy for dealing with unexpected or research-related deaths, or animal waste. All staff should be made aware of these procedures, and the focus shall be on biosafety.

F.9 Environmental enrichment

F.9.1 Housing should provide a complex, warm, comfortable and stimulating environment. It is important to provide animal-to-animal and animal-to-human socialization and areas for the animal to retreat from one another or seek refuge. Boredom in animals should be alleviated wherever possible.

F.9.2 Pens and cages should contain:

- a) one litter tray per cat plus one extra tray;
- b) ample shelf room for resting (see 7.6.5.1 and 7.6.5.3.2.3);
- c) objects suitable for climbing and claw care; and
- d) vertical structures for cats to scent-mark on.

F.9.3 Toys and activity feeders are recommended as enrichment.

F.9.4 Appropriate hard items should be provided for dogs to chew as this prevents gingivitis and periodontal disease.

F.9.5 An animal behaviour specialist should be consulted to develop a suitable enrichment programme.

F.10 Hygiene

F.10.1 Cleaning and disinfection

F.10.1.1 Animal housing and exercise runs should be kept clean so that the comfort and health of the animals can be maintained, and disease-controlled.

F.10.1.2 Faeces and residual food should be removed and kennels and units cleaned daily. Animals should only be returned to dry housing and should not be restricted to wet areas.

F.10.1.3 Fresh substrate and litter should be provided.

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F.10.1.4 Before new animals are introduced or after any disease outbreak, animal housing should be thoroughly cleaned and disinfected, with a facility-approved disinfectant.

F.10.1.5 Disinfectants and cleaning agents should be chosen on the basis of their suitability, safety, and effectiveness. Manufacturer's instructions for the mixing and use of these agents should be followed. Package inserts and technical data on agents should be filed for reference purposes.

F.10.2 Litter trays

F.10.2.1 Cats should be provided with litter trays with a minimum dimension of 300 mm × 400 mm. Sufficient suitable litter material, such as commercial cat litter, should be provided, and litter trays cleaned and disinfected daily (see also F.9.2 (a)).

F.10.2.2 Since cats can be substrate specific, changes of litter substrate should be done gradually, with some of the litter trays that contain the original substrate always available until all the cats use the litter trays that contain the new substrate.

F.10.3 Waste disposal

F.10.3.1 Waste disposal should be done in accordance with the requirements of the municipal authority.

F.10.3.2 Solid waste (faeces, food residues and litter) should be disposed of in a manner that is suitable and acceptable to the person-in-charge.

F.10.4 Pest and vermin control

F.10.4.1 Every effort should be made to control unwanted pests, flies and wild rodents.

F.10.4.2 Some pesticides and rodenticides are toxic to animals and should be used with care by designated trained staff. All animals should be excluded from treated areas until the poison programme is completed and no residual toxicity levels remain.

F.10.5 Emergency procedures (see 6.3.2.9.8)

F.10.5.1 An adequate plan should be provided to cover all emergencies. All staff should be made aware of this plan and procedures involved.

F.10.5.2 Emergency contact numbers should be clearly displayed.

F.10.5.3 Emergency firefighting equipment should be placed at easily accessible areas, and emergency exits clearly marked.

F.10.5.4 Emergency power supply should be available.

F.11 Breeding management

F.11.1 Breeding systems

F.11.1.1 Optimizing fecundity with breeding colonies that supply animals for research purposes is recommended since this reduces the numbers of animals being used for breeding purposes. There should be safeguards to assure the welfare of breeding animals, systems that assure that breeding occurs at the correct stage of maturity, and that subsequent breeding does not compromise health or general welfare.

F.11.1.2 Ultrasound scanning is the recommended method of pregnancy diagnosis. It allows earlier detection of pregnancy with an accurate prediction of parturition to within 2 d to 3 d.

F.11.1.3 All breeding stock should be subjected to regular veterinary clinical health examinations.

F.11.1.4 The following are commonly used breeding systems:

- a) observed mating system;
- b) harem mating system; and
- c) artificial insemination (including the use of frozen semen).

F.11.2 Selection of breeding stock

F.11.2.1 Behaviour assessments should be part of the selection process.

F.11.2.2 When selecting suitable replacement breeders, animals that show minimal amount of fear and distress on exposure to novelty and isolation, and fastest recovery rates to these situations, should be selected.

F.11.2.3 Animals exhibiting appreciable levels of fear, long recovery times, abnormal behaviour, stereotypic behaviour, and reluctance to interact with handlers should not be considered for future breeding programmes.

F.11.3 Dogs — Care of the whelping bitch

F.11.3.1 Pre-parturient bitches should be moved to the whelping kennel area at least two weeks before the expected date of parturition. They may be pair- or group-housed to provide them with companionship.

F.11.3.2 Bitches will seek solitude and privacy to whelp their young and need to be moved into the whelping kennel 2 d to 3 d before whelping, but within sight and sound of other pre-parturient and lactating bitches.

F.11.3.3 In the period 12 h to 24 h before whelping, the bitch's temperature will decrease from the normal $38,5\text{ °C} \pm 0,5\text{ °C}$ to $36\text{ °C} \pm 0,5\text{ °C}$. The temperature should be taken twice daily for several days before expected whelping in order to anticipate the whelping time.

F.11.3.4 Bitches should be provided with the opportunity for short periods of exercise from approximately 3 d post-partum.

F.11.4 Cats — Care of the periparturient queen and litter up to three weeks of age (see 7.6.5.3.3.1)

F.11.4.1 The pregnant queen should be housed singly only in late pregnancy, and preferably within the last week. She will seek isolation in a confined space for the act of parturition and for a period during the early suckling of her young. A quiet, private area should be provided for this purpose.

F.11.4.2 Where there is confinement within cage environments, additional exercise should be provided for the queen, on a daily basis. This will include contact with humans and ancillary play equipment.

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F.11.5 Cats — Queen and litter from three weeks of age to weaning

See 7.6.5.3.3.2.

F.11.6 Minimizing losses and care of the newborn

Factors to be considered in minimizing losses of the newborn are the following:

- a) the previous reproductive performance of the individual mother;
- b) health status of the mother;
- c) age of the mother;
- d) hygiene in the whelping area;
- e) close management of the parturition process;
- f) staff education, training and expertise;
- g) care of the whelping mother;
- h) care of the newborn;
- i) congenital defects;
- j) early separation of the young from the mother; and
- k) poor individual records on sires and dams.

F.12 Socialization, habituation and training

F.12.1 Socially housed animals should be compatible. There are variations in temperament, and sympathetic husbandry practices shall take this into account.

F.12.2 For dogs, pens and runs should have sufficient length for nervous or subordinate dogs to retreat.

F.12.3 Solid partitions might be necessary to prevent injuries and to provide some privacy, especially for the periparturient bitch. As dogs are inquisitive by nature, especially regarding their surroundings, this can be catered for by providing lower solid partitions towards the front of the pen, to increase the field of view.

F.12.4 Dogs make extensive use of chews, especially if they are food-flavoured. However, monopolization can occur by more dominant or aggressive animals, and this should be monitored.

F.12.5 Advice on behavioural aspects should be sought from a qualified animal behaviour specialist.

F.12.6 Positive interactions with humans should take place throughout the animal's life and the principles of positive reinforcement should be applied.

F.13 Grouping

F.13.1 Groups should be selected and monitored for social compatibility. Any aggression shall be addressed immediately.

F.13.2 Attempts should be made to place animals in established, compatible groups within the confines of a study protocol.

F.13.3 Behavioural assessments should be done on new arrivals and used as a guide for group allocation and development purposes.

F.14 Balancing supply and demand

F.14.1 In order to minimize surplus animals, all possible measures should be taken to ensure that the supply of animals does not exceed demand.

F.14.2 To match supply and demand, good communication is necessary between the person-in-charge, researchers and users. Where surpluses occur, it is necessary to review the causes and rationalize the programme(s).

Scientific requirements for single sex or specific weight range animals should be questioned and justified.

F.14.3 Animals that display undesirable behaviour or fear-related behaviour should be considered first for short-term terminal procedures, rather than for long-term use.

F.14.4 Relocation of aged ex-breeding animals to other establishments for use in scientific procedures is not acceptable.

F.14.5 Any rehabilitation of surplus animals should be done as early in life as possible.

F.14.6 Production planning is critical in colony management.

F.15 Transport

F.15.1 Attention shall be given to relevant standards for transport, i.e to IATA Regulations, which will provide useful guidelines on transport and caging.

F.15.2 Animals shall be transported in the shortest practicable time. Routes should be planned and provision made for emergencies.

F.15.3 Animals shall be given an acclimatization period of at least 7 d to 10 d after extended transport, and 3 d for relocation on site or between pens. At least 14 d should be allowed for acclimatization before the start of scientific studies.

F.15.4 Animals should not be physically carried as a means of on-site transport. Dogs may be walked or lead-walked or transported in crates or trolleys. Cats should be carried in cages or baskets. Where animals have to be carried, their full body weight should be adequately supported.

F.15.5 Any vehicle designed and used for transport should:

a) protect the animal(s) from injury;

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- b) have non-slip floors;
- c) provide easy and safe access for the operator;
- d) be well-ventilated and temperature-controlled;
- e) protect against unauthorized release, or escape, of animals; and
- f) be supplied with clean, secure cages or carry baskets for cats and small dogs, and with separate compartments or partitions for larger dogs.

F.15.6 The driver should carry an emergency veterinary kit in the vehicle and be trained in its use, or be accompanied by trained personnel. A full list of emergency contact numbers and a cell phone should be available in the vehicle.

F.16 Identification and records

F.16.1 It is good practice in any establishment to be able to uniquely identify each individual animal.

F.16.2 The method of identification should not be invasive, painful or cause mutilation or adverse reaction. Non-invasive methods are recommended, especially for pre-weaned animals.

F.16.3 An assessment should be done of whether permanent marking of individual animals is necessary.

F.16.4 Subcutaneous microchip implants (minimally invasive) are the recommended, most widely used and most satisfactory method of permanent identification. Tattooing is not recommended, and if performed, local or general anaesthesia should be used.

F.16.5 Each animal should ideally have a "Personal File" as a permanent life case history.

F.16.6 Non-invasive methods of identification include the following:

- a) diagrams of coat and colour patterns;
- b) felt-tip pen marking of the ear(s); and
- c) collars and tags.

F.16.7 Invasive methods of identification include the following:

- a) microchips;
- b) tattoos (on the ear, flank or lip); and
- c) ear-notching.

F.17 Handling and restraint

F.17.1 One of the most important ways of minimizing stress in animals in laboratory facilities is to ensure regular handling and attention. All animals should experience adequate socialization with humans during the primary socialization period, combined with habituation and training studies. Where applicable, this process should continue throughout the animal's life.

F.17.2 It is essential to understand species-specific typical behaviour and communication systems to be able to interpret signals and respond accordingly. Consistent and empathetic approach is recommended.

F.17.3 Stroking an animal is beneficial. Direct eye contact and potentially threatening body postures should be avoided.

F.17.4 Temporary restraint can be stressful to animals, but habituation and training can reduce this. Duration of restraint should be as short as possible.

F.17.5 Abnormal or prolonged restraint should be soundly justified. Slings and metabolic cage restraint will require a careful and patient acclimatization process, and their use should be monitored at all times.

F.17.6 Positive reinforcement methods should be used to train animals to tolerate restraint.

F.17.7 Before procedures begin, time should be allowed for the training of animals to accept procedure rooms, metabolic cages, restraint devices and stressful procedures. Any animal that does not respond or settle quickly with training should be considered unsuitable.

F.18 Procedures

F.18.1 Administration of substances

It is a common research procedure to administer substances to an animal, and the route is often dictated by the scientific procedure. Administration of substances should be through the least aversive route possible, and with the least potential for adverse effects over the whole procedure.

F.18.2 Removal of body fluids

F.18.2.1 Assessment should be made as to whether the collection of fluids, other than blood and urine, is necessary. The least invasive method(s) should be used.

F.18.2.2 Potential refinements for the removal of body fluids that involve repeated collections are vascular catheterization for blood and urinary catheterization for urine.

F.18.3 Metabolic cages

F.18.3.1 Metabolic cages should not be used unless they are scientifically constructed and their use is soundly justified, especially if alternative methods that require less confinement and isolation are available.

F.18.3.2 Justification for the use of metabolic cages should be included in a cost-benefit analysis and should be authorized by an ethics committee.

F.18.3.3 The dimensions, design and construction of metabolic cages should be such as to minimize the impact on animal welfare.

F.18.3.4 Consideration should be given to habituating animals to metabolic cages, including grid flooring, during procedural training.

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F.18.4 Telemetry

F.18.4.1 Telemetry procedures and husbandry for animals fitted with telemetry devices should be refined to reduce pain and distress.

F.18.4.2 Where jackets or collars are required, gradual habituation and monitoring for adverse effects is required.

F.18.5 Anaesthesia, analgesia and perioperative care

F.18.5.1 Where animals are expected to experience pain and distress during and after procedures, appropriate anaesthesia and analgesia should be administered.

F.18.5.2 Pre-anaesthetic evaluation of the animal(s) health status and condition should be carried out.

F.18.5.3 Appropriate anaesthetic and analgesia regimens should be researched and used.

F.18.5.4 Protocols for dealing with pain and chronic discomfort should be in place before a study begins.

F.18.5.5 Review of the perioperative care plan should be done regularly, and any improvements should be made, including recommendations for future protocols.

F.18.6 Recognizing and monitoring adverse effects

F.18.6.1 Wherever possible, pain should be avoided, alleviated and prevented.

F.18.6.2 Before commencing a scientific procedure, a list of likely or expected adverse clinical effects should be drawn up with mitigating treatments.

F.18.6.3 Retrospective assessments of clinical, surgical and perioperative procedures and treatments should be conducted.

F.18.6.4 Staff involved with perioperative care should be appropriately trained, able to recognize adverse reactions, and should take appropriate action to alleviate conditions.

F.18.6.5 Records of adverse reactions, treatments and any animal welfare related issues should be recorded.

F.18.7 Euthanasia

F.18.7.1 In accordance with the Animals Protection Act, 1962 (Act No. 71 of 1962), when an animal is found to be severely sick, injured or suffering and, in the opinion of the veterinarian, the animal would not respond to treatment and it would be cruel to keep it alive, the veterinarian shall forthwith destroy the animal or order its destruction. A signed veterinary report and certificate might be required in such cases.

F.18.7.2 Where euthanasia is required, a humane method ensuring rapid and painless induction, unconsciousness, and death is essential. Intravenous injection of an overdose of sodium pentobarbitone (200 mg/kg) is the recommended method for dogs and cats.

F.18.7.3 Neuromuscular blocking agents should never be used alone to put animals to death.

F.18.7.4 It should be ensured that all staff chosen to carry out euthanasia on animals are competent, willing, mentally prepared, and empathetic to carry out the process.

F.18.7.5 Euthanasia should always be carried out in an area separate from all other animals.

F.19 Long-term use

F.19.1 When maintaining animals for long periods in laboratory facilities, consideration should be given to the adverse effects of long-term housing, husbandry, and procedures.

F.19.2 Additional consideration should be given to ensure the behavioural, social and physiological needs of these animals. Additional resources might be necessary.

F.19.3 The criteria for euthanasia should be considered, including the welfare cost of long-term confinement.

F.20 Adoption or re-homing

F.20.1 The possibility of adoption or re-homing, as an alternative to euthanasia, should be considered. Only healthy animals, and those not suffering any adverse effects from their experiences in the laboratory, should be considered.

F.20.2 The adoption or re-homing process should consider all the issues relating to animal welfare, the capabilities of the new owner, and the new environmental conditions.

F.20.3 Adopted or re-homed animals will have to undergo a new socialization, habituation and training programme in their new habitat. An animal behaviour specialist's advice should be considered in this process.

F.21 Training of personnel

F.21.1 Staff should be aware of their responsibilities, should respect the animals and shall have experience in their handling. Handling methods should be standardized.

F.21.2 Staff levels should be adequate for the size of the facility, and to manage all breeding colonies and husbandry practices.

F.21.3 In setting staff levels, the time for important human/animal social interaction requirements should be taken into account, especially for single-housed animals. This should include regular handling time.

F.22 Animal welfare considerations

F.22.1 Long-term single housing and social isolation can lead to behavioural problems with dogs and cats and should only be used as a last resort for an aggressive animal. Additional daily human contact is necessary in such cases.

Single housing should only be permitted for established welfare reasons such as pregnancy, periparturition, injury, veterinary or disease isolation, or where group housing is incompatible with justifiable scientific objectives.

F.22.2 There should be sufficient staff time allowed daily for social interaction with animals. This time should be in addition to normal routine cleaning and feeding. A continuing education

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programme is essential to assist and inform staff of novel developments and practices in animal management, behaviour and welfare.

F.22.3 Suitable veterinary, hospitalization, and isolation or quarantine facilities should be provided.

F.22.4 Adding to the pen complexity (by providing enrichment devices, toys, raised platforms, heating, runs, tactile contact, and view of conspecifics, etc.) is beneficial to animal welfare.

F.22.5 Chewing is an important behaviour and items should be provided which meet this need.

F.22.6 Where metabolic cages or crates are to be used, their minimum dimensions should be agreed upon, and justified, for the specific animal(s) to be confined.

F.22.7 When instituting a stress reduction programme, it is important to treat each animal as an individual. An individual cage record card can assist staff in understanding each animal's preferences and dislikes.

F.22.8 Some animals that come from isolated environments might prefer NOT to be housed with other animals. This is especially true for cats. Provision for single housing and social interaction with humans should be made in such cases.

F.22.9 Negative interactions, stressful handling, and restraint procedures should be minimized.

F.23 Records

Comprehensive records should be kept of each animal held in the facility and all animals should be identifiable.

F.24 References

See bibliography.

Annex G

(informative)

Care and management of laboratory animals — Fish

G.1 General

G.1.1 Fish and aquaculture research focuses mainly on the areas of environmental or ecological pollution, conservation, protection of marine and estuarine habitats, health and husbandry of food fishes, and molecular, genetic and toxicology studies.

G.1.2 Fish need to be maintained in controlled environments and emphasis shall be placed on limitation of stress, humane handling and animal welfare aspects.

G.1.3 Researchers and all persons involved with the advancement of scientific knowledge through the use of fish need to understand and appreciate these animals, their ecosystems and their requirements for essential life processes.

G.2 Availability of fish species

Choice of species depends on research demands, and the selection of the correct or suitable model. Ease of maintenance, suitable housing facilities and trained staff are important considerations. Certain fish species are aggressive, and require more space, specialized diet, social compatibility, specialized housing, or life support systems.

G.3 Capture and acquisition

G.3.1 General

G.3.1.1 Irrespective of the purpose for which live fish are being collected, a strict ethic of habitat conservation and humane treatment of the animals should be observed.

G.3.1.2 Collection of large numbers of animals from breeding populations, and unacceptable collection techniques and habitat destruction should be avoided.

G.3.1.3 Sampling equipment and strategies should be designed to minimize "by-catch" and non-target species.

G.3.1.4 The choice of collection method should take into account the welfare of the animals, worker safety, research objectives, seasonal conditions, and the type of habitat.

G.3.2 Representative samples

The study design usually dictates the number of animals required, but the principle of only taking the smallest number of animals required should be observed.

Poor handling of large numbers of captured fish can result in high and unnecessary mortalities.

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G.3.3 Collection of imperilled species

G.3.3.1 Imperilled species applies to those animals officially listed as threatened or endangered. It also applies to those animals identified as candidates for listing. It is important to know if an area or a habitat supports imperilled species and how to identify them.

G.3.3.2 Collection of imperilled species should be avoided, unless the research being conducted is to the benefit of that species, and the necessary permits have been obtained.

G.3.3.3 Collection techniques, such as injurious or lethal ichthyocides, are not recommended.

G.3.3.4 Translocation of imperilled species might require specialized equipment and conditions. This will include the transport used for their return into the wild. Biosafety and biosecurity issues should be considered.

G.3.4 Wild fish and captive-bred fish

G.3.4.1 Fish caught in the wild may be captured by the research team (with necessary Conservation or CITES permits) or be bought from suppliers. Collection techniques shall be declared. Where dead fish, fish products and eggs are collected, it is wise to ascertain the disease status and disease transmission risks before transporting to the laboratory.

G.3.4.2 Captive-bred fish are available from hatcheries and other laboratory supply houses, aquaria, and hobbyists.

G.3.4.3 Applicable animal welfare laws shall be considered.

G.3.5 Killed and museum specimens

G.3.5.1 The collection of fish from natural populations, for preservation, is necessary for:

- a) understanding basic biology, evolution and life history;
- b) documenting and recording biodiversity;
- c) establishing reference collections;
- d) environmental impact assessments and ecological surveys (voucher specimens); and
- e) geographic variation and delineation of new species.

G.3.5.2 Each animal collected should serve as many types of study as possible to reduce the total numbers collected to a minimum.

G.3.5.3 The use of piscicidal (ichthyocides) agents for capture should take into account the effects on other species in the environment. Conservation authority approval needs to be obtained, and justification for their use be provided to the AEC.

G.3.5.4 Fish should be put to death by recognized euthanasia methods before immersion in formalin.

G.3.6 Acquisition of hatchery fish

G.3.6.1 Fish should come from hatcheries with defined and acceptable health status, and preferably known genetic history.

G.3.6.2 Hatcheries that regularly supply fish to laboratories should be encouraged to develop husbandry and management practices consistent with those of the laboratories.

G.4 Transport

G.4.1 Contingency plans should be drawn up for any vehicle breakdowns during transport.

G.4.2 Important considerations are water quality, oxygen, temperature, and ammonia levels. Cooling the water will reduce the metabolic rate and thus reduce the amount of ammonia excreted into the water as well as the oxygen requirement. Fish excreta lowers pH value levels.

G.4.3 Fish may be taken off food for 2 d to 3 d before being transported. They will then have voided their digestive tract contents and will not excessively foul transport water.

G.4.4 Transport boxes are usually made of cardboard lined with polystyrene (styrofoam) panels for insulation and protection. Ideally, fish should be packed into square-bottomed plastics bags that provide better protection. Bags should be half-filled with original aquaria housing water. The bag should be inflated to balloon capacity with oxygen and sealed off with an elastic band. Newspaper should be used to isolate bags from each other and to absorb any excess water. Spiny fish have the capacity to puncture a plastics bag, and shall be transported in more durable containers.

G.4.5 The packing method in G.4.4 can sustain fish comfortably for 12 h to 24 h. Express shipping should always be used to limit transport time to less than 24 h.

G.5 Quarantine and acclimatization

G.5.1 The primary purpose of quarantine is the containment and isolation of newly introduced fish and associated biota for a period of observation, testing, and acclimatization. This will also ensure acceptable health status (freedom from unwanted disease and parasites) and suitability for reliable research studies. Any structural and management changes shall be approved by the IBSC to ensure continued biosafety standards.

G.5.2 It is recommended that a quarantine manual be developed with accompanying SOPs for dealing with all quarantine requirements and contingencies. The responsibilities of the manager and personnel should be defined.

G.5.3 Personnel working in the quarantine facility should be adequately trained in quarantine procedures and disease recognition.

G.5.4 A quarantine period of 30 d is recommended for new introduction shipments. Access to the quarantine area(s) should be controlled and limited to designated staff only, and suitable signage should be displayed. Protective clothing (caps, gloves, goggles, gumboots, gowns or coats) should be provided. Suitable wash facilities should be provided, i.e. elbow-operated handbasins, paper towel dispenser and approved detergent or surgis scrub dispensers.

G.5.5 Each quarantine tank should have its own independent filtration system, and each tank should be specifically designated to a particular shipment or introduction of fish. Separation of shipments of fish of unknown health status is vital. All tanks should be clearly marked and

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comprehensive tank records should be kept. Separation distance between tanks should be such as to prevent splashing of water from one tank to another.

G.5.6 Fish arriving in transport bags should be acclimatized by placing the bags in the tank water to equilibrate temperatures between the bags and the tank water (normally for 30 min). Bags may be clamped to the side of the tanks so that they can be opened for aeration. To reduce stress, the bags should be handled as little as possible, and lighting levels kept low. Transfer of fish should be done gently, using appropriate fine mesh nets.

G.5.7 On entering the quarantine facility, fish should be inspected for any abnormalities and external lesions. Appropriate samples should be taken. The lesions may be routinely treated with approved broad-spectrum antibiotics, antiparasitics, and antifungal agents.

G.5.8 Progeny of fish which breed in quarantine may be moved to another tank but should remain in the quarantine facility for the duration of the quarantine period.

G.5.9 Dead fish should be removed immediately and postmortem or laboratory testing (or both) should be carried out.

G.5.10 All designated cleaning and other equipment should remain in the quarantine area.

G.5.11 Lighting provision should be adequate for inspection purposes. Dimmer systems may be incorporated.

G.5.12 Aquaria should have at least one side made of clear material for inspection of fish, and should be fitted with lids to prevent fish jumping out, and to minimize splash or spillage.

G.5.13 The coving in the holding room should be at least 150 mm high to contain any accidental water spills (for example, from tank ruptures).

G.5.14 All wastewater, when discharged from the facility, should enter directly into an approved municipal sewer system. It might be necessary to treat wastewater. This water should be chlorinated for a period of 20 h with an available chlorine level of not less than 200 mg/L.

G.5.15 Solid waste should be disposed of by an approved method such as incineration, or be removed by a waste collection company that conforms to the regulations promulgated under the Environment Conservation Act, 1989 (Act No. 73 of 1989).

G.6 Quality assurance and SOPs

Quality assurance (QA) plans and SOPs are required as essential tools in the management and operation of animal research facilities. They provide guarantees that systems are operating and functioning efficiently, thereby promoting valid research data obtained via consistency in repeated procedures, limiting unnecessary replications, reducing the overuse of animals, and the protection of animals and staff. QA plans and SOPs form the basis of essential and effective training programmes.

G.7 Animal welfare considerations

G.7.1 General

G.7.1.1 Capture techniques (seines and traps, gill nets, ichthyocides (piscicides), electrofishing, hooks and spears) should be justified by the AEC as to their suitability to minimize the possibility of capture distress and pain.

G.7.1.2 All statutory laws and regulations covered by the Marine and Coastal Management of the Department of Environmental Affairs and Tourism and the Animal Protection Act, 1962 (Act No. 71 of 1962) should be observed. There should be as little as possible or no disturbance of natural habitats. The use of experienced personnel is essential.

G.7.1.3 Appropriate attention should be given to study design and procedures whilst ensuring the humane treatment of study subjects.

G.7.1.4 There are essential differences between fish and other vertebrates that are critically important for the conduct of scientifically valid research, such as the following:

- a) mortality patterns differ in fish, especially in egg survival;
- b) fish field research, or early life stage research, requires much larger numbers; and
- c) handling, housing, care and maintenance requirements differ from those for vertebrates.

G.7.1.5 The AEC has the responsibility to carry out scientific reviews that guarantee the effective, efficient and valid design of protocols, studies, animal welfare considerations and veterinary treatments. It is recommended that the AEC carries out regular inspections and audits of research facilities.

G.7.2 Pain and distress

G.7.2.1 Researchers should take great care to avoid inducing stress and pain in fish research subjects, especially on a prolonged basis.

G.7.2.2 In fish, any deviations from normal homeostasis, will result in stress.

G.7.2.3 Appropriate use of anaesthetics and analgesics in procedures that can cause pain is essential.

G.8 Aquatic facilities and housing

G.8.1 Security and access

Access to aquatic facilities should be designed, and controlled, to minimize traffic through the area(s). Access should be restricted to authorized personnel only.

G.8.2 Types of systems (Flow through, recirculation and static)

G.8.2.1 The type of system used should depend on the appropriateness for the species to be housed and should consider factors such as water quality.

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G.8.2.2 Correct water management is critical for the wellbeing and survival of fish held in aquaria. All water should be analyzed before setting up an aquarium to establish the current pH value, ammonia, nitrates, calcium, etc., in the water.

G.8.3 Effective environmental monitoring and control of tanks

G.8.3.1 All architectural and engineering specifications and drawings should be available on site for staff responsible for the running and maintenance of the facility. Records of maintenance programmes and schedules should be kept.

G.8.3.2 The staff responsible for the facility management and for fish care should be available 24 h per day for routine and emergency needs.

G.8.4 Water quality and management

G.8.4.1 General

G.8.4.1.1 A sound environmental monitoring system is essential, and the complexity should be designed to adequately monitor and control the water management system(s).

G.8.4.1.2 All monitoring equipment should be regularly serviced and calibrated.

G.8.4.1.3 Detailed records should be kept of all maintenance and repairs for retrospective analysis.

G.8.4.2 Temperature

G.8.4.2.1 The health, nutrient requirements, performance, reproduction and survival of fish is dependent on water temperature, and optimum temperature criteria vary for different species.

G.8.4.2.2 Gradual equilibration of water temperature is crucial when transferring, shipping, breeding and acclimatizing fish, and when changing tank water. An optimal temperature variation is 1 °C/h.

G.8.4.3 Oxygen and supersaturation

Temperature variation affects the saturation of gases, especially oxygen. There is less dissolved oxygen at higher temperatures. In closed aquaria, sudden large increases in temperature are very detrimental to the fish and shall be avoided by appropriate regulating mechanisms.

G.8.4.4 pH value

G.8.4.4.1 pH values of between 6,5 and 9,0 are desirable. pH value has multiple effects on dissolved gases and metals in the water, as well as on oxygen uptake by fish. The value will also affect organic acids, phosphates, and the ratio of non-ionized-to-ionized ammonia in the water.

G.8.4.4.2 Fish vary in their tolerance to pH values at various stages of their lifecycle. pH values of 6,5 and above are required for normal breeding and reproduction.

G.8.4.5 Salinity, alkalinity and hardness

G.8.4.5.1 General

The total amounts of solid materials dissolved in the water is important since fish need specific elements to carry out vital biochemical processes and depend on their surrounding medium for these requirements.

G.8.4.5.2 Salinity

Salinity is the amount of dissolved salts in the water that affects the density of the water and temperature requirements of certain species. When transferring fish, salinity changes should be gradual, and shall be monitored.

G.8.4.5.3 Alkalinity

Alkalinity is the measure of the acid-neutralizing capacity of the water. Bicarbonates, carbonates, borates, phosphates, and other anions contribute to alkalinity (milli-equivalents per litre). Adequate alkalinity ensures buffering of acid metals and proper functioning of biofilters.

G.8.4.5.4 Hardness

Hardness is the measure of mineral content (primarily calcium, magnesium, and other divalent cations). Appropriate hardness might decrease stress toxicity due to dissolved metals and ammonia.

G.8.4.6 Nitrogenous compounds and toxic agents

G.8.4.6.1 Nitrogen is present in water as gas, nitrates, nitrites and ammonia.

G.8.4.6.2 Ammonia is the most toxic inorganic nitrogen produced by fish and by heterotrophic bacteria. A safe level for ammonia is considered to be 0,02 mg/L. Nitrite toxicity can occur in recirculation water systems and causes methaemoglobinaemia and ultimately hypoxia.

NOTE Combined excess levels of ammonia and nitrites are responsible for "new tank syndrome" where fish stay near the surface of the water gasping for breath, feed less, sometimes show behavioural abnormalities and can result in death by hypoxia or secondary diseases.

G.8.4.6.3 All chemical products should be stored well away from aquatic housing and the water supply. Chemical storage facilities should be lockable and secure.

G.8.4.6.4 Where there is reason to believe hazardous materials have entered the water system(s), such system(s) should be immediately isolated and tested.

G.8.4.7 Water supply

Four main processes are necessary to maintain optimum water quality in closed systems:

- a) biological filtration – removal of bacteria and nitrification processes;
- b) mechanical filtration – removal of particulate;
- c) chemical filtration – granulated activated carbon, foam fractionation and ion exchangers; and
- d) disinfection – ozonization and UV light treatment.

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G.8.5 Engineering, design and materials

The correct materials (for example, concrete, plastics, fibre, glass and glues) shall be chosen for the plumbing and for the tanks. These should not contribute products that are toxic to the tank or to the holding water container. Construction materials should not contain copper, nickel, cadmium, or brass.

G.8.6 Mechanical and electrical requirements

G.8.6.1 All electrical systems should be professionally installed and should comply with the relevant national standards.

G.8.6.2 Extension cords and system(s) overloading should be avoided.

G.8.6.3 Electrical components and equipment should be located outside the splash zone, and in moisture-proof enclosures. Seawater is corrosive and has a high electrical conductivity, therefore adequate precautions, such as insulation, inspection and preventive maintenance, should be taken.

G.8.6.4 Machinery that produces noise and vibration should be isolated from the areas housing fish (see 7.5.4.5).

G.8.6.5 Critical systems, including pumps, should be duplicated to ensure that failures cause minimal disruption. An emergency power supply should be available at all times. This should be tested regularly to ensure proper and efficient functioning.

G.8.7 Lighting

G.8.7.1 Both photoperiod and light intensity, and the variations for each species, are important.

G.8.7.2 Most species do well at a 12 h/12 h light/dark cycle, although some tropical fish prefer a 10 h/14 h light/dark cycle.

G.8.7.3 Fluorescent lighting is most commonly used. Full spectrum lighting may be used over tanks.

G.9 Husbandry and breeding

G.9.1 Record keeping

Detailed SOPs, daily records and checklists should be developed for the maintenance and care of all fish species, for sanitation and cleaning procedures of tanks and rooms, and for the maintenance of equipment.

G.9.2 Density and carrying capacity

Each species should be housed at a density that optimizes the wellbeing of the fish while meeting study parameters. Where necessary, the ideal environment will have to be developed using performance-based criteria such as growth rate.

G.9.3 Food, feeding and nutrition

G.9.3.1 Fish are one of the most efficient animals in converting food nutrients into body tissue. They are poikilotherms, and excrete waste products efficiently and require little energy for support and transport.

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G.9.3.2 Proteins make up 60 % to 70 % of fish tissue on a dry weight basis. Vitamins and minerals should be given in proper ratios to ensure a well balanced diet. The research being undertaken might determine these requirements. Other important factors are the stability of the food in the water and the levels of resultant pollution. Overfeeding and pollution of the tank water should be avoided.

G.9.3.3 Fish food should only be purchased from sources approved by the person-in-charge, and in accordance with the standards required.

G.9.3.4 Feed bags should be labelled and the label should include manufacture date and provide detailed analysis information. Bags should be stored at optimal or recommended temperatures, in a designated feed storage area. All bins should be lidded and sealed.

G.9.4 Broodstock and breeding

G.9.4.1 Holding systems and environmental conditions should be appropriate for the species being held.

G.9.4.2 Attention should be given to environmental cues for the maintenance, stimulation, or manipulation of endogenous reproductive rhythms.

G.9.5 Environmental enrichment

Environmental enrichment should be provided (see 7.6.6). Some species require plants, refuge areas or gravel to exhibit natural behaviour.

G.10 Health and disease control

G.10.1 Fish health programme

G.10.1.1 All facilities should have a fish health-monitoring programme, and fish should be observed daily for signs of illness and abnormal behaviour.

G.10.1.2 A health management programme should focus on early diagnosis and identification of causal agents, and rapid initiation of control measures. It might be necessary to remove sick fish from the aquarium and collect specimens for laboratory examination. Each tank should have a fish mortality record.

G.10.1.3 Dead fish should be incinerated.

G.10.1.4 Drug and chemical administration to fish and to water tanks should be subject to the approval of the person-in-charge or a veterinarian. Records should be kept of all treatments.

G.10.2 Injuries and handling

G.10.2.1 Fishes should be fasted before handling or manipulation.

G.10.2.2 Personnel that handle fish should be trained and be experienced to reduce handling injuries.

G.10.2.3 Handling should be reduced to the minimum essential episodes. Fish should be protected from bright direct lighting or rapid changes in lighting while they are being restrained.

G.10.2.4 Fish should not be kept in the open air for more than 30 s.

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G.10.3 Vermin control

Surveillance should be maintained for the presence of unwanted vermin, and a control programme shall be undertaken if required.

G.11 Laboratory studies using fish

G.11.1 Fish should not be held indefinitely without an AEC approved protocol.

G.11.2 The manager of the facility should be responsible for maintaining a comprehensive and up-to-date record of all fish and studies in the facility, and should ensure compliance with all quality assurance programmes. Routine auditing of facilities is recommended.

G.11.3 Key personnel shall be listed.

G.12 Study procedures

G.12.1 Statistical design

G.12.1.1 The number of fish subjects required for an investigation will depend on the research questions being asked. Field and laboratory studies require very different study statistical designs. Field and early life stage studies require very large numbers of fish subjects.

G.12.1.2 The use of adequate and valid numbers to establish variance and assure reliability of results is essential to prevent needless repetition and fish overuse. A statistician should be consulted to develop study designs that have the appropriate statistical power to accomplish sound objectives.

G.12.2 Restricted movements

Every effort should be made to provide fish held in restricted environments with as non-stressful an environment as possible. Restraints, as required by research design, should be justified and approved by the AEC.

G.12.3 Surgery

G.12.3.1 Surgery should be performed by personnel with appropriate training and expertise.

G.12.3.2 Surgical sites should be prepared in a sterile manner which also minimizes tissue damage and contamination.

G.12.3.3 During prolonged surgery, water quality should be maintained at a high level. Water for anaesthesia should be sourced from the holding tank.

G.12.3.4 Appropriate anaesthetics should be used to provide adequate safety margins, predictable results and rapid recovery. Under field conditions, anaesthetic effects vary with temperature, water quality, species, size of fish, and life stage.

G.12.3.5 Any incisions should avoid the lateral line and should follow a longitudinal axis.

G.12.3.6 Suture materials should be strong, inert, non-hygroscopic and be placed with atraumatic needles.

G.12.4 Administration of compounds and devices

G.12.4.1 If a treatment compound is to be administered orally, the dose rate should not exceed 1 % of body weight (1 mL/100 g).

G.12.4.2 Intramuscular injections may be made into the large dorsal epaxial and abdominal muscles, but should avoid the lateral line and ventral blood vessels.

G.12.4.3 Intraperitoneal injections should avoid penetrating the abdominal viscera.

G.12.4.4 Implanted materials should be biocompatible and aseptic, and be implanted using sterile techniques.

G.12.4.5 Care should be taken to introduce the suture needle in spaces between the scales.

G.12.5 Marking and tagging

G.12.5.1 Marking methods are used mainly for movement assessments, for management and for population dynamics. It is important to consider the effects of marking and tagging on fish health, physiology, behaviour and survival.

G.12.5.2 Methods used include DNA markers, fin clipping, electrocauterization or freeze-branding (under general or local anaesthesia), tattooing, radio telemetry, radioisotope injections (13C, 15N, or 34S), and tagging. Any proposed method should be justified and approved by the AEC.

G.12.5.3 Release of fish back into the wild should comply with Nature Conservation regulations. Fish should be in good health, be able to function normally in the new environment, be released back to the natural home range, and not introduce any pathogenic agents into the surroundings.

G.12.6 Collection of body fluids and tissue

G.12.6.1 Results obtained from careful collection and examination of blood and tissue samples are often critically important for research results. Sterility under field conditions is not always possible, and procedures should be designed to minimize contamination.

G.12.6.2 Sedation or anaesthesia should be used for restraint in collection or cannulation purposes as physical restraint will affect the serum glucose and hormonal levels.

G.12.6.3 Blood is collected via three main routes; *viz.* cardiac puncture, venous puncture and caudal vein bleeding. Tissue is collected after fish have been appropriately anaesthetized, or humanely put to death.

G.12.7 Endpoints and monitoring

G.12.7.1 Study endpoints, other than death of the study subjects, should be developed, clearly outlined, and understood, unless death is required and justified by an AEC approved protocol.

G.12.7.2 Researchers should eliminate, mitigate or minimize potential pain and distress whenever possible. The use of a pilot study should be considered where appropriate monitoring parameters have not yet been defined.

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G.12.7.3 In any study where there is expected morbidity and mortality, the criteria for endpoints and early euthanasia should be defined. A list of parameters should be established to permit objective assessment of health, pain and distress status.

G.12.7.4 The frequency of monitoring should allow for the timely removal of fish, before severe morbidity occurs.

G.12.8 Negative reinforcement

When using negative reinforcement modalities in fish, pilot studies (see O.7) and literature searches should be used to establish the least invasive method of obtaining a consistent response.

G.12.9 Exercise to point of exhaustion

Studies that involve swimming to the point of exhaustion, often in conjunction with negative reinforcement, should be justified and approved by the AEC. Strict attention should be given to continuous monitoring and the elimination of undue distress.

G.12.10 Environmental extremes

Studies that involve the exposure of fish to environmental extremes should be justified and approved by the AEC. Endpoints should be clearly defined.

G.12.11 Genetically modified fish

Genetically modified fish (transgenic fish) should not be permitted to enter the food chain.

G.13 Holding and disposition of study fish

It is the responsibility of the researcher and institution to ensure that all regulations and permits pertaining to the fish being captured, transported, held in captivity and under study are complied with.

NOTE Work with many species is regulated by the provisions of CITES. The Organization for Economic Cooperation and Development (OECD) is concerned with toxicological testing methods for human health. Eco-toxicological test methods, including testing on fish, and OECD guidelines are available for reference purposes at <http://www.oecd.org>.

G.14 Dangerous aquatic fish and safety considerations

G.14.1 It is important to note the human safety aspects when working with fish of unknown origin and health status. The risk of zoonoses should be assessed. Even the smallest fish can have defence mechanisms that can be dangerous to humans. Diseases can be transmitted to and from the fish.

G.14.2 Feeding and handling are high risk activities. Emergency procedures to cover these activities should be outlined and understood by trained staff. When working with dangerous fish, it is advisable that two persons be present at all times.

G.14.3 Traumatogenic fish are those that cause injury, mainly via bites, stings, electric shock, and punctures. The stings of certain venomous fish can cause serious cardiovascular effects and irreversible cardiac arrest. In many cases, secondary bacterial infection that could develop from the stings can be serious.

G.14.4 All staff working in designated laboratories should follow safety protocols and guidelines set out in safety manuals with regard to biohazards, chemicals, radioisotopes, and dangerous animals.

G.15 Anaesthetics and analgesics

G.15.1 General

G.15.1.1 Anaesthetics and analgesics should be used in a regulated, judicious and appropriate manner to effect pain relief sedation, immobility, loss of equilibrium and controlled loss of consciousness for surgery, handling, transport and capture.

G.15.1.2 Most commonly used anaesthetics and analgesics are the substances that can mix easily with water, and allow minimal physical restraint once fish have been placed in the solution.

G.15.1.3 For recovery, fish are placed in a well-oxygenated anaesthetic-free environment. Jaw tone returns before opercular activity. It might be necessary to manually propel fish through the water to force water through the mouth and gills.

G.15.1.4 The following anaesthetics and their recommended dosage can be used on fish:

- a) **Tricaine methanesulfonate** (MS-222) is absorbed rapidly via gill diffusion. The anaesthetic dose range varies for different species and is between 50 mg/L to 200 mg/L. Aeration shall be provided in the anaesthetic solution as hypoxia is a potential side effect.
- b) **Benzocaine and benzocaine hydrochloride** is a highly insoluble powder that shall first be dissolved in ethanol or acetone. A stock solution of 100 gm/L is generally made up and concentrations of 25 mg/L to 200 mg/L are used.
- c) **Metomidate** is often used as a transport sedation at a dose rate of 0,06 mg/L to 0,20 mg/L. For most fish, anaesthesia is achieved at a dose rate of 2,5 mg/L to 5,0 mg/L. Induction is rapid, but recovery can be prolonged in accordance with the time the fish are exposed to the drug.
- d) **Ketamine hydrochloride** provides excellent anaesthesia in teleosts (bony fish) when injected intramuscularly at a dose of 60 mg/kg to 80 mg/kg. In most cases, induction takes 10 min to 20 min and provides 10 min to 20 min of surgical time. A Ketamine (12 mg/kg) and Xylazine (6 mg/kg) mixture is used for sharks.

G.15.2 Stages of anaesthesia in fish

Stage 1 – erratic swimming, excitement, some loss of equilibrium, disorientation, increased respiration, some loss of tactile response and reduced activity.

Stage 2 – loss of equilibrium, slow swimming movements with loss of direction, and decreased respiration.

Stage 3 – complete loss of equilibrium, slow swimming and respiration, and reduced responses to stimuli. Surgical plane is reached when fish are unable to swim, respiration is shallow, and there is no response to stimuli. Cessation of opercular movements.

Stage 4 – spasmodic over distension of opercules and cardiac failure.

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G.16 Euthanasia

Where euthanasia in fish is required, the following two-step process should be used wherever possible:

- a) anaesthesia to loss of equilibrium; followed by
- b) physical or chemical method to cause brain death.

MS-222 is recommended at 500 mg/L followed by an acceptable method to ensure brain death.

G.17 References

See bibliography.

Annex H

(informative)

Care and management of laboratory animals — Horses

H.1 General

Throughout the world, horses are kept in a wide variety of situations. Many breeds exist, adapted to a broad range of environmental conditions. Horses have been domesticated for several thousand years and man has developed a profound understanding of their needs. These needs relate to their physiological and behavioural requirements such as grazing, exercise and them being distinct herd animals. It is important to cater for those needs in order to provide adequate housing and care to horses.

H.2 The environment

H.2.1 General (outdoors)

H.2.1.1 Horses can be acclimatized to adverse climatical conditions. For reasons of providing standardized research environments, these animals are often stabled in environmentally-controlled facilities.

H.2.1.2 If horses are housed outdoors, they require proper shelter from the sun, wind, rain and other adverse weather conditions. They also require access to a dry, well-drained area for rest. This area should be large enough to accommodate all horses lying down at the same time.

H.2.2 Temperature (indoors)

H.2.2.1 Horses housed indoors should generally be maintained at room temperatures between 16 °C and to 22 °C.

H.2.2.2 In special cases, for example, when housing very young or recovering animals, higher room temperatures than those indicated (see H.2.2.1) might be required. Gradual acclimatization should be done before moving them outdoors after they have adapted to indoor conditions.

H.2.2.3 Room temperature should be monitored daily, preferably by continuous recording. A less costly alternative is the use of a maximum and minimum thermometer that is examined and re-set daily. However, since this does not indicate how long the room was held at a particular temperature, knowledge of which is extremely important, the use of a thermograph is therefore recommended. The temperature of the microenvironment should also be monitored.

H.2.2.4 Occasionally, optimal temperature for the laboratory animal is not the most comfortable for personnel. However, human preferences should not compromise the study requirements or the health and comfort of the animal.

H.2.3 Relative humidity

Humidity control is an important consideration for laboratory animals since it contributes to the variability of research models. For horses, a relative humidity in the range of 55 % ± 15 % is acceptable. Most animals prefer a relative humidity of approximately 60 %, but can tolerate a range of 40 % to 70 % as long as it remains relatively constant and the temperature range is appropriate.

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H.2.4 Ventilation

H.2.4.1 Ventilation influences temperature, humidity, and gaseous and particulate contaminants in the animal cage and holding room. The design of the building ventilation system should permit the maintenance of these parameters within acceptable limits.

H.2.4.2 The actual ventilation rate required varies with age, sex, species, stocking density, frequency of cleaning, quality of incoming air, ambient temperature and humidity, and the type of construction of primary and secondary enclosures, among other factors.

H.2.4.3 Draft-free air exchanges in the range of 10 exchanges to 15 exchanges per hour are commonly recommended for rooms that contain horses under conventional housing conditions.

H.2.4.4 Differential pressures can be used to inhibit the passage of pathogenic material between rooms. Higher pressures are used in clean areas, as opposed to dirty or biohazardous ones, in order to minimize contamination. Generally, a differential pressure of 2,5 mm to 5,0 mm mercury is maintained.

H.2.5 Lighting

H.2.5.1 The three characteristics of light that can influence laboratory animals are intensity, quality, and photoperiod. The lighting should provide good visibility and uniform, glare-free illumination. Light tubes, which imitate the spectrum of sunlight, are commercially available and their use is recommended.

H.2.5.2 Where natural lighting is not used, light and dark periods should be at least 6 h each per day.

H.2.5.3 Photoperiod is probably the most influential of light characteristics on laboratory animals. It is suggested that if a change occurs in an animal's photoperiod, then no experiments should be conducted with that animal for at least a week. If a longer light phase is interrupted by a shorter dark phase, there are few significant effects. However, if the reverse occurs, endogenous rhythms can be significantly skewed. This is one reason why automatic timers should control light cycles in all animal rooms. Timer function should be monitored or hooked into an alarm system. A daily cycle of 12 h dark:12 h light is usual. Additionally, any windows in an animal room should be capable of being blacked out.

H.2.6 Noise

Sudden irregular noises create more disturbances in horses than continuous or predictable sounds. Noise cannot be eliminated from an animal unit but care should be taken to minimize the generation of sudden extraneous audible and ultrasound noise in the vicinity of animals.

H.2.7 Vibration

Vibration stability is important for the maintenance of a constant study environment for sensitive animals. Therefore, animal holding and test rooms should be located away from areas such as a cagewash, major circulation corridors where racks are frequently in transit, mechanical rooms, and elevator shafts. Vibration studies should be performed to determine how best to achieve the maximum allowable vibration levels as determined by instruments and animals to be used in the area.

H.3 Animal care and health

H.3.1 General

H.3.1.1 Unless there is good husbandry, veterinary or scientific justification for individual housing, animals should be maintained in compatible sociable groups. These groups should remain stable. Horses are herd animals which depend on social contact and will show severe stress reactions if separated from their group. If individual housing is required, the animals should at least have visible contact with conspecifics.

H.3.1.2 When housed in barn-type accommodation, there should be sufficient feeding space, water points and resting areas to avoid confrontations. Where space to avoid conflict is not available (as in most indoor housing), visual barriers should be provided.

H.3.1.3 Horses respond well to positive food reinforcement such as the provision of barley or lucerne. Low stress handling can be achieved by competent, calm and confident personnel within an environment that is designed to assist such efforts.

H.3.2 Bedding material

H.3.2.1 Absorbent bedding material such as straw or wood shavings should be added to interior pens to provide a clean, comfortable and dry surface. A minimum average layer thickness of at least 15 cm of bedding material is recommended.

H.3.2.2 Bedding may be non-nutritive, but should be non-toxic, absorbent and comfortable. Resinous wood shavings, especially cedar, are not suitable for use as laboratory animal bedding. Pine shavings should be avoided for the same reason, although they are not as toxic as cedar.

H.3.2.3 Floors designated to accommodate horses require special caution. Care should be taken that the floors are specifically designed for horses, should prevent injuries (i.e. have no floor drains or other significant unevenness or holes), provide secure footing and be comfortable.

H.3.3 Food and water (see table H.1)

H.3.3.1 Potable water should be supplied to animals in sufficient quantity and be presented in a manner that an animal can use. Water receptacles should be sited to avoid fouling, while still being accessible to young foals. Tap water might be sufficient for conventional housing facilities.

H.3.3.2 Where large numbers of animals are maintained in pens, it is important to ensure that there are sufficient feeding and watering stations to avoid undue competition. Restricted feeding of groups of horses is not recommended as it leads to competition. Consequently, the information in table H.1 is recommended.

Table H.1 — Minimum recommended requirements for feeding and watering equipment for a 500 kg horse

1	2	3
Feeding and watering equipment	Size or number of troughs and feeders	Throat height cm
Watering facilities	1 trough/20 horses	20 to 80
Grain feeders	(30×20×15) cm ³	60 to 90
Hay feeders	(60×50×60) cm ³	60 to 106

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H.3.3.3 Particularly when horses are allowed to graze on pastures, animal attendants and veterinary personnel should be aware that horses might ingest material other than normal feedstuffs.

H.3.3.4 An individual animal's nutrient requirements are affected by many factors. Young animals generally need increased amounts of many nutrients. Reproduction places many demands on female animals, and nutrient requirements are very high in gestating and lactating animals. Environmental temperature and humidity can also affect food intake and nutrient needs.

H.3.3.5 All feed should be clean, free of contaminants or pests, palatable, fresh and sufficient for the animal's needs. The selected food should be a balanced diet that provides all required nutrients.

H.3.3.6 The technique of Body Condition Scoring (BCS) should be learned by all animal attendants to assess whether or not the diet of the animals in their care is maintaining the animals in good body condition.

H.3.4 Cleaning

H.3.4.1 Routine cleaning and maintenance, and a high standard of hygiene are essential for good husbandry. Suitable and institutionally approved cleaning agents and procedures should be applied.

H.3.4.2 The facilities should be designed to support manure removal, cleaning and disinfection.

H.3.4.3 Decisions on the frequency of cleaning should be based on the housing system, type of animal, stocking densities, and the ability of ventilation systems to maintain suitable air quality.

H.3.4.4 Fly, tick and other pest populations should be regularly monitored and appropriate control measures be applied when indicated.

H.3.5 Environmental enrichment

H.3.5.1 Environmental enrichment should be something that simulates normal equine behaviour such as foraging. A food dispensing apparatus, for instance, has been shown to be effective in maintaining normal behaviour in stabled horses. Horses also enjoy sand and mudbaths, or rubbing against poles and trees.

H.3.5.2 Exercise, social contact with other horses and grooming are other forms of environmental enrichment. Even training with positive reinforcement can be considered an environmental enrichment effort when it stimulates aspects of normal equine behaviour.

H.3.5.3 Since horses are particularly prone to developing stereotypic behaviour (such as wood chewing and cribbing), environmental enrichment and adequate handling and feeding regimens are critical factors for maintaining the horse's welfare.

H.3.6 Animal accommodation (see table 12)

H.3.6.1 Equine housing facilities should provide suitable access and restraining devices to allow animals to be inspected, caught or moved as necessary.

H.3.6.2 Under natural conditions, horses spend long periods of foraging (grazing) while moving considerable distances. The housing management should take cognizance of this fact and provide access to an outside exercise area whenever possible. Such outside areas should provide sufficient shade and water to accommodate the needs of all animals present at the time.

H.3.6.3 If horses are maintained over long periods, hoof trimming should be part of the animal management programme.

H.3.6.4 Pens should be of sturdy construction to contain the animals securely and should be designed and maintained to prevent horses from becoming trapped or injuring themselves.

H.3.6.5 Space allowances for horses vary greatly depending on animal size, breed, gestation status, climate conditions, etc. In general, pens should be large enough to allow all horses to lie comfortably on a dry and bedded area. During transport or when in other pens where horses are kept for short periods, enough space should be allowed for all animals to stand comfortably.

H.3.6.6 For specific purposes (for example, immediate post-operative care or metabolic studies) it might be justified to restrict the available space or other aspects of the primary enclosure (or both). Such studies should state these conditions clearly in the proposal to the AEC for it to be approved.

H.3.7 Breeding

H.3.7.1 Foaling mares should be familiar with their environment and their handlers and should be allowed to give birth with minimum interference. Animal attendants should be familiar with normal birth and should be able to recognize problems. Assistance in foaling should, if necessary, be provided under veterinary supervision.

H.3.7.2 Newborn foals require adequate nutrition and a high level of hygiene. Mothers and their offspring should be disturbed as little as possible.

H.3.7.3 Detailed records should be kept of pedigrees as well as of fertility and rearing success.

H.3.8 Animal identification

H.3.8.1 General

H.3.8.1.1 The most important considerations in choosing a marking technique concern its effect on the behaviour, physiology and survival of the animal. Any technique that causes an adverse effect on the animal is not only inhumane, but is likely to distort the data being collected, resulting in meaningless and misleading results.

H.3.8.1.2 For registered horses, the breed registry will determine the acceptable methods of identification. However, in choosing an acceptable marking technique, the researcher should consider the nature and duration of restraint, the amount of tissue removed or damaged, whether or not pain, if inflicted, is momentary or prolonged, and whether the risk of infection and abscessation is minimal.

H.3.8.2 Permanent marking

H.3.8.2.1 The physical description of permanent signs such as colour, markings, breed and position of hair whorls and scars, is one of the most common identification systems for horses.

H.3.8.2.2 Ear-notching is not recommended for horses.

H.3.8.2.3 Microchips are widely used to uniquely identify animals. New generation microchips even allow for the measuring of body temperature or the storage of animal data on the chip.

H.3.8.2.4 Ear-tags of a suitable size for horses are widely available and often used. More than two tags per ear is considered excessive. When reapplying tags, the operator should use the pre-existing hole(s) in the ear.

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H.3.8.2.5 Tattoos on one or both lips may be used. Tattooing should be carried out by an experienced operator, using properly maintained equipment and good hygienic practice.

H.3.8.2.6 Hot-iron or freeze-branding might be required under some circumstances. If these methods of identification are required, adequate anaesthesia or sedation and analgesia should be provided.

H.3.8.3 Semi-permanent marking

H.3.8.3.1 A patch of hair or patterns may be shaved, clipped or cut with a pair of scissors. Such marks generally last from one week to four weeks (depending on the stage of the hair cycle) and can be used on any colour horse.

H.3.8.3.2 Hoof branding might also aid identification.

H.3.8.4 Temporary marking

Horses are often marked with marking sticks that leave a strip of colour on the coat. This is easily applied but only lasts for several days, and then it can be reapplied.

H.3.9 Handling

H.3.9.1 Horses should be handled quietly, with care and patience, to avoid injury, pain or distress. A properly equipped handling area should be available to facilitate the treatment of horses. All handling and restraining equipment should be positioned and used humanely and with regard to the horse's natural movement, temperament and physical capabilities. Persons working with horses should avoid sudden movements or actions that might frighten the animals, and should always be alert and observant towards the behaviour displayed by the horses.

H.3.9.2 All tack and equipment should be maintained in good operating condition. All halters, leads and lariats, and other materials used to restrain or handle horses should be equipped with a method of quick release in case a horse becomes entangled in the equipment. Chutes used to restrain horses should have break-out walls to assist horses that go down during handling.

H.3.9.3 A very important factor in the management of horses is the actual caretaker, who should be comfortable working with horses, be alert and observant, and handle horses gently, but effectively. The grooming is an excellent opportunity to establish and maintain a bond between the caretaker and the horse, and allows for an opportunity to examine the horse's body.

H.3.9.4 Horses should be groomed several times a week, particularly when they are shedding, and the hooves shall be cleaned daily.

H.3.10 Records

Regular monitoring of health and reproductive data, and keeping detailed records thereof, is essential to ensure that problems are identified at an early stage so that corrective action can be implemented to minimize any potentially adverse welfare effects on the animals. This form of monitoring and assessment is of particular importance in herds, where large numbers of animals are maintained, or where there is a high animal turnover.

H.4 References

See bibliography.

Annex I
(informative)

**Care and management of laboratory animals —
Non-human primates (baboons and vervet monkeys)**

I.1 General

The information supplied in this annex is confined to baboons (*Papio ursinus*) and vervet monkeys (*Chlorocebus aethiops* previously *Cercopithecus aethiops*), which are commonly used in biomedical research locally. It is possible that in wildlife research other primate species might also be maintained and expert advice shall be sought for these.

I.2 International guidelines (see table I.1)

International guidelines are often created in the United States of America (USA) or Europe, with no or minimal consultation of countries with wild primate populations.

Recommendations for cage sizes vary internationally and some are currently under review.

Comparisons between guidelines cannot always be made directly. For example, the minimum floor area and cage height for a single primate cage is less in the USA than in Europe (Reinhardt *et al.* 1996) (see bibliography). When a mate is added, this floor area does not have to be enlarged in Europe, but has to be doubled in the USA.

Table I.1 — A selection of published guidelines

1 Weight of primate kg	2 Minimum floor area m ²				3 Minimum height cm			
	A ^a	B ^b	C ^c	D ^d	A ^a	B ^b	C ^c	D ^d
1 to 3	0,14 to 0,27	0,35	0,24	0,36	51 to 76	75	60	80
3 to 5	0,39	0,50	0,4	0,36	76	80	80	80
5 to 7	0,39	0,70	0,4	0,36	76	85	80	80
7 to 9	0,39	0,90	Nd ^e	0,56	76	90	Nd ^e	80
9 to 15	0,39 to 0,54	1,10	Nd ^e	0,56	81	125	Nd ^e	120
15 to 25	0,72	1,50	Nd ^e	0,80	91	125	Nd ^e	120
>30	1,50	1,50	Nd ^e	2,00	116	Nd ^e	Nd ^e	210

^a Guide for the care and use of laboratory animals. National Academy of Sciences, 1996.
^b Council of Europe Guidelines, 2007.
^c Tsukuba Primate Centre, Japan, 2004.
^d National Code for the Handling and Use of Animals in Research, Education, Diagnosis and Testing of Drugs and Related Substances in South Africa, 1990.
(See bibliography for complete titles.)
^e No data.

Some European contentions are disputed in the USA (Schapiro *et al.* 2000) (see bibliography).

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European centres have relatively little exposure to African primates. There is considerably more experience with African primates in the USA, although macaques dominate as the species of choice. As a result, housing needs are generally less well-defined for African primates. This is of importance because species-specific considerations are critical. There are also significant differences in temperament among the species.

I.3 General guidelines

Rigid guidelines can inhibit more creative approaches and there should be enough flexibility to accommodate alternative methods. For example, absolute insistence on a certain cage dimension can prevent the simultaneous use of several, smaller interconnected units, which might create a much more complex environment and be of more benefit to animal welfare. This, however, shall not be used to reduce the total amount of space available to the primates. The onus should be on the facility to show that their approach is valid.

I.4 Special considerations regarding vervet monkeys

Some research conducted on vervet monkeys in an indoor facility (Seier *et al.* 2004) (see bibliography) has so far shown the following:

- a) No serious abnormal behaviour (for example, self-injurious or bizarre behaviour) in adults, even in smaller single cages (unlike with other species). Stereotypic behaviour does occur.
- b) Enrichment and complexity can reduce stereotypic behaviour to below that seen in much larger cages.
- c) Significant inter-individual differences in the display of stereotypic behaviour.
- d) Urinary cortisol levels in laboratory-housed vervet monkeys are below those assumed to be indicators of stress in macaques. However, urinary cortisol should not be used alone as an indicator of stress but in conjunction with behavioural observations as well as other observations.
- e) Vervet monkeys are more arboreal than some related species and baboons (Gebo DL and Sargi EJ 1995, Ankel-Simons 2000) (see bibliography).

I.5 Special considerations regarding the differences between baboons and vervet monkeys

I.5.1 The following are significant differences between baboons and vervet monkeys:

- a) size and weight;
- b) social structure and troop size; and
- c) baboons are more terrestrial (Ankel-Simons 2000) (see bibliography).

I.5.2 One similarity with vervet monkeys is that baboons can develop stereotypic and other abnormal behaviour (Kessel and Brent 2001) (see bibliography).

I.5.3 Presently neither are systematically captive-bred locally

I.6 General considerations

I.6.1 Primates should only be housed in single cages of minimum dimensions (see table 13) for the absolute shortest period of time required to successfully conclude the study.

I.6.2 In some situations, a smaller cage size than recommended might be desirable for very short periods of time (i.e. days rather than weeks) such as during intensive care, transport or quarantine. Professional judgement should be applied in these cases.

I.6.3 Facilities are strongly encouraged to exceed the minimum cage sizes. However, cage size alone does not determine abnormal and stereotypic behaviour. This is also observed in zoo animals housed in large enclosures (Hosey and Skyner 2004) (see bibliography).

I.7 The environment

I.7.1 General

I.7.1.1 Although baboons and vervet monkeys occupy diverse habitats, environmental conditions during indoor housing are standardized. Recommendations for environmental conditions in this standard apply to indoor housing since, during outdoor housing, the primates are exposed to environmental elements to a large extent.

I.7.1.2 Animals housed outdoors are invariably exposed to a wide range of temperatures, but protection against extremes should be provided. This includes shade or shelter against sunlight and high temperatures, or a heat source for very cold conditions. Shelters should also protect the animals from rain. Local weather patterns should be taken into account, i.e. from which direction the rains generally come, and prevailing winds.

I.7.1.3 Low-ranking or otherwise weaker members of a group might be ousted from shelters. Care should be taken to provide enough shelters for all members of a group, considering social composition and dynamics. Outdoor housing is generally not suitable for research purposes other than behavioural studies, and is usually used to maintain breeding and stock animals in communal enclosures.

I.7.2 Temperature

I.7.2.1 A good temperature range for baboons and vervet monkeys is 22 °C to 26 °C, but size, age and condition of the animals have to be considered.

I.7.2.2 Monitoring and recording of temperatures, and other considerations, is as recommended in the relevant section for rodents (see L.2.2).

I.7.3 Relative humidity

A humidity range of 30 % to 70 % is considered acceptable for most Old World species.

I.7.4 Ventilation

I.7.4.1 Ventilation influences temperature, humidity, and gaseous and particulate contaminants in the animal cage and holding room. The design of the building ventilation system should permit the maintenance of these parameters within acceptable limits.

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I.7.4.2 The actual ventilation rate required varies with age, sex, species, stocking density, frequency of cleaning, quality of incoming air, ambient temperature and humidity, and the type of construction of primary and secondary enclosures, among other factors.

I.7.4.3 Draft-free air exchanges in the range of 15 exchanges to 20 exchanges per hour are commonly recommended for rooms that contain primates under conventional housing conditions.

I.7.4.4 Differential pressures can be used to inhibit the passage of pathogenic material between rooms. Higher pressures are used in clean areas, as opposed to dirty or biohazardous ones, in order to minimize contamination. Generally, a differential pressure of 2,5 mm to 5,0 mm mercury is maintained.

I.7.5 Lighting

I.7.5.1 The lighting should provide good visibility and enable complete routine inspection of animals and animal rooms.

I.7.5.2 No conclusive studies are available regarding optimal light intensity for non-human primates. Primates have developed and reproduced normally through several generations in indoor environments lit by fluorescent lights. However, the lack of exposure to sunlight has to be compensated with dietary vitamin D to prevent deficiency of this micronutrient.

I.7.5.3 A spectrum and light intensity suitable for humans is often presumed to be suitable for non-human primates as well. Light tubes, that imitate the spectrum of sunlight, are commercially available but should be used with caution.

I.7.5.4 A 12 h photoperiod supports normal biological functions. Care should be taken that the dark phase is not accidentally interrupted by light thereby causing significant disruption of the circadian rhythm. Therefore, automatic timers should control light cycles in all animal rooms. Timer function should be monitored or hooked into an alarm system. A daily cycle of 12 h dark:12 h light is usual.

I.7.6 Noise

Sudden irregular or loud noises can be highly stressful. Noise cannot be totally eliminated from an animal unit but care should be taken to minimize the generation of sudden extraneous audible and ultrasound noise in the vicinity of animals.

I.7.7 Vibration

I.7.7.1 Care should be taken not to site animal rooms near a constant or intermittent source of vibration.

I.7.7.2 Vibration stability will be of greater concern if the animal facility is located on the upper levels of a building rather than at ground level because of structural considerations.

I.8 Animal care and health

I.8.1 General

Unless there is good husbandry, veterinary or scientific justification for individual housing, animals should be maintained in compatible sociable groups. However, socializing of unfamiliar adult primates is associated with a considerable risk of injury and stress to the animals. Such socializing shall be approached with a great degree of caution and sensitivity. Size and complexity of

enclosures, social density, as well as size, age, sex and temperament of individuals are some key determinants for success. Even groups that are initially stable do not necessarily remain so.

I.8.2 Bedding

Although bedding absorbs urine, in primate care it is more frequently used to enable foraging. The bedding material, such as wood shavings and corncob from a reputable source, should be non-toxic and free from chemical or biological contamination. Although ingested bedding can form intestinal obstruction (Seier *et al.* 2005) (see bibliography), this is quite rare, and the benefits outweigh the disadvantages.

I.8.3 Food and water

I.8.3.1 Uncontaminated potable water should be supplied to animals in sufficient quantity and be presented in a manner that an animal can use. In communal housing it is important to ensure that there are sufficient feeding and watering stations to avoid undue competition.

I.8.3.2 An individual animal's nutrient requirements are affected by many factors. Young animals generally need increased amounts of many nutrients. Reproduction places many demands on female animals, and nutrient requirements are very high in gestating and lactating animals. Environmental temperature and humidity can also affect food intake and nutrient needs.

I.8.3.3 All feed should be clean, free of contaminants or pests, palatable, fresh and sufficient for the animal's needs. The selected food should be a balanced diet, that provides all required nutrients.

I.8.3.4 Most primates use a wide variety of food items in the wild, and much of their daily activity is devoted to foraging. Therefore, feeding has behavioural dimensions beyond providing the requisite amounts of nutrients and, in captivity, has an important environmental enrichment function.

I.8.3.5 Standardized pelleted diet is fed in the laboratory, which will affect species-specific behaviour of food usage in the laboratory as compared to in the wild. It has been established that the nutritional balance of a dry diet, such as pellets, is not significantly altered by the addition of up to 50 % (wet basis) of food items with high water contents (NRC 2003) (see bibliography). Therefore, the easiest and most scientifically sound way of providing food diversity is by supplementing the diet with fruit and vegetables. It is important to note that the food type plays an important role in dental health.

I.8.4 Cleaning

I.8.4.1 Routine cleaning and maintenance, and a high standard of hygiene are essential for good husbandry. Suitable and institutionally approved cleaning agents and procedures should be applied.

I.8.4.2 Decisions on the frequency of cleaning should be based on the housing system, type of animal, stocking densities, and the ability of ventilation systems to maintain suitable air quality.

I.8.4.3 Rooms that contain no bedding, and where animals defecate and urinate on the floor or in drop pans, should be cleaned daily. Bedding in communal cages should be changed three times per week, at which time the surfaces should also be sanitized.

I.8.4.4 A disinfectant with a suitable spectrum and activity should be used regularly. There should be a documented pest-control programme, which will depend on the circumstances and location of different facilities.

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I.8.5 Environmental enrichment

I.8.5.1 Different primate species occupy diverse habitats, live in different social structures and use different food items. All these result in specific behavioural needs. International standards have been established, mainly for Asian macaques and a number of New World primates, whereas comparatively little data is available for African primates.

I.8.5.2 New research constantly provides new insights, and the status of environmental enrichment is very much in flux. Recommendations should therefore be flexible, so as to incorporate new ideas and replace practices shown to be ineffective.

I.8.5.3 The following minimum standards should apply:

- a) environmental enrichment is obligatory, and every facility that maintains non-human primates should have a documented enrichment plan; and
- b) the plan should list all environmental enrichment measures taken in such facilities, as well as their frequency and duration.

I.8.5.4 The environmental enrichment plan should address the following broad issues:

- a) social contact;
- b) foraging;
- c) manipulanda (for example, food puzzles);
- d) cage complexity and structure;
- e) food as enrichment;
- f) sensory enrichment (for example, music and mirrors); and
- g) a rationale for the enrichment plan based on published data (where available), own observations or own documented research (even if unpublished).

I.8.6 Animal accommodation (see 7.6.8)

I.8.6.1 The quality of space is as important as its quantity, and cage enrichment and social interaction are considered to be of more value than simple floor space allocation.

I.8.6.2 Morphological considerations of baboons and vervet monkeys that affect cage size are the following:

- a) The average weight and height of vervet monkeys

Adult male:	5,50 kg
Adult female:	3,60 kg
Crown rump length (sitting height):	adult male: 49 cm
Crown heel length (standing height):	adult male: 76 cm

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The following points should be also be taken into account when deciding on cage sizes for vervet monkeys (see 7.6.8.2):

- 1) the actual sitting height of vervet monkeys is lower than the crown rump length since the monkeys never sit routinely fully extended but lean forward on their arms;
- 2) the cage should be high enough to enable installing a resting perch, so that a fully-grown monkey can sit comfortably under as well as on top of the resting perch; and
- 3) if the animals are kept in cages that comply with the minimum dimensions only (see table 13) for extended periods, mobile exercise cages of about two to four times the dimensions of the home cage should be available for rotational use amongst animal room inhabitants.

b) The average weight and height of baboons

Adult male:	30kg to 35 kg, up to 44 kg
Adult female:	17 kg to 20 kg
Crown rump length (sitting height):	80 cm
Crown heel length (standing height):	about 1,25 m

When deciding on cage sizes for baboons, the same considerations as for vervet monkeys (see 1.8.6.2 (a)(1) to (3) (inclusive)) apply, except that a much larger cage (see table 13) is required, with a higher and larger resting perch.

1.8.7 Breeding

Both baboons and vervet monkeys have been bred successfully and over successive generations in indoor and outdoor facilities. Considerable literature in the field is referred to. All breeding systems including pair, harem or multi-male/multi-female social groups are generally successful, and the choice depends on the type of facility, and housing system (see 1.8.6). Breeding in the wild occurs naturally in social groups, but it carries the most risk in terms of aggression, injury and infant mortality. Therefore, pair-breeding might be preferable in some situations.

Offspring raised by their mothers should not be weaned earlier than 10 months to 12 months as early weaning is associated with the development of behavioural problems.

1.8.8 Animal identification

1.8.8.1 General

1.8.8.1.1 The most important considerations in choosing a marking technique concern its effect on the behaviour, physiology and survival of the animal. Any technique that causes an adverse effect on the animal is not only inhumane, but is likely to distort the data being collected, resulting in meaningless and often misleading results.

1.8.8.1.2 In choosing an acceptable marking technique, the researcher should consider the nature and duration of restraint, the amount of tissue removed or damaged, whether or not pain, if inflicted, is momentary or prolonged, and whether the risks of infection and abscessation is minimal.

1.8.8.2 Permanent marking

1.8.8.2.1 Microchips are widely used to uniquely identify animals. New generation microchips even allow for the measuring of body temperature or the storage of animal data on the chip. Due to the

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large gauge of the implanting needle, the implantation of microchips should always be performed under general anaesthesia in sterile conditions.

I.8.8.2 Tattooing on the inner thigh or chest is another less expensive way of positively identifying individuals. A tattoo should be applied under anaesthesia.

I.8.8.3 Semi-permanent marking

A patch of fur or patterns may be shaved, clipped or cut with a pair of scissors.

I.8.8.4 Temporary marking

A felt-tip marker may be used for marking an ear or tail. This is easily applied but only lasts for 1 d to 2 d, and then it can be reapplied.

I.8.9 Handling

I.8.9.1 Non-human primates in the laboratory have to be handled regularly for a variety of examinations and procedures. Considering the sensitivity of primates to such interventions, handling should be carried out with great care and skill to minimize stress and injury to both animals and handlers.

I.8.9.2 During husbandry procedures, such as transferral to another cage, animals can be trained to enter a smaller transport cage voluntarily. For certain procedures anaesthesia is required, which is usually administered by intramuscular injection in the home cage. However, non-human primates are increasingly trained by positive reinforcement to co-operate voluntarily with minor procedures such as blood sampling.

I.8.9.3 Substances can be administered orally in food or food treats. However, in some cases, gavaging might be necessary. If gavaging has to be frequent, this might have to be without anaesthesia, but this is obviously not possible with animals the size of baboons.

I.8.9.4 Physical handling should only be done by highly skilled people.

I.8.10 Records

I.8.10.1 Permanent electronic or manual records should be kept for every individual animal. These should contain health, reproductive and other relevant data, and should be updated as necessary.

I.8.10.2 A person who is suitably qualified or experienced (or both) should inspect all non-human primates daily, and should record any deviation from normal health and behaviour on dated and consecutively numbered log sheets. The numbering system enables cross-referencing with the individual records where necessary. A log sheet might record observations of the entire colony, and one page might be enough for several days if nothing unusual needs to be recorded.

I.9 References

See bibliography.

Annex J (informative)

Care and management of laboratory animals — Pigs

J.1 General

Pigs are raised in many ways, including outdoor pasture systems, mixed indoor-outdoor systems, and indoor systems, the latter which prevails in research facilities. Each has its own advantages and disadvantages, for both animals and operators, but it is common to all systems that the animal wellbeing is largely determined by the amount and quality of care and the operator's understanding of the animals.

Pigs are very sensitive and intelligent animals that require special attention and care to ensure their physical and behavioural wellbeing.

J.2 The environment

J.2.1 General

J.2.1.1 Pigs can be acclimatized to relatively narrow margins of climatical conditions. For reasons of providing standardized research environments, these animals are often stabled in environmentally-controlled facilities.

J.2.1.2 If pigs are housed outdoors, they require proper shelter from the sun, wind, rain and other adverse weather conditions. They also require access to a dry, well-drained area for rest. This area should be large enough to accommodate all pigs lying down at the same time.

J.2.2 Temperature (see table J.1)

J.2.2.1 Temperature in pig housing systems is possibly the most important factor determining the comfort level of pigs. Temperature requirements for pigs vary with their age and size, and the environmental conditions of the housing system.

Table J.1 — Thermal comfort zones for pigs of various ages

1	2	3
Stage of growth	Weight of pig kg	Range of zone °C
Piglet	< 6	34 to 24
Weaner	7 to 25	32 to 18
Grower	26 to 50	25 to 15
Finisher	51 to 100	25 to 15
Breeding stock	>100	21 to 10

J.2.2.2 The use of focal heat sources (for example, heating lamps) is recommended for piglets and weaners. This allows the animals to choose between areas of different temperatures. It is, however, important that such heat sources provide sufficient heated space to accommodate all animals in a pen at the same time.

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J.2.2.3 The way piglets or pigs lie in relation to each other and to the heat source is a reliable indicator of the suitability of the ambient temperature. Pigs resting comfortably in the heated area indicate optimal temperatures. Piglets crowding, piling on each other, or shivering indicates low temperatures. Piglets avoiding the heated zone or resting at its perimeter indicates high temperatures.

J.2.2.4 In special cases, for example, when housing very young or recovering animals, higher room temperatures than those indicated (see table J.1) might be required. Gradual acclimatization to outdoor conditions needs to be made before moving them outdoors.

J.2.2.5 Room temperature should be monitored daily, preferably by continuous recording. A less costly alternative is the use of a maximum and minimum thermometer that is examined and reset daily. However, since this does not indicate how long the room was held at a particular temperature, knowledge of which is extremely important, the use of a thermograph is therefore recommended. The temperature of the microenvironment should also be monitored.

J.2.2.6 Occasionally, optimal temperature for the laboratory animal is not the most comfortable for personnel. However, human preferences should not compromise the study requirements or the health and comfort of the animal.

J.2.3 Relative humidity

Humidity control is an important consideration for laboratory animals since it contributes to the variability of research models. For pigs, a relative humidity in the range of 55 % ± 15 % is acceptable. Most animals prefer a relative humidity of approximately 60 %, but can tolerate a range of 40 % to 70 % as long as it remains relatively constant and the temperature range is appropriate.

J.2.4 Ventilation

J.2.4.1 Ventilation influences temperature, humidity, and gaseous and particulate contaminants in the animal cage and holding room. The design of the building ventilation system should permit the maintenance of these parameters within acceptable limits.

J.2.4.2 The actual ventilation rate required varies with age, sex, species, stocking density, frequency of cleaning, quality of incoming air, ambient temperature and humidity, and the type of construction of primary and secondary enclosures, among other factors.

J.2.4.3 Draft-free air exchanges in the range of 10 exchanges to 15 exchanges per hour are commonly recommended for rooms that contains small livestock under conventional housing conditions.

J.2.4.4 Differential pressures can be used to inhibit the passage of pathogenic material between rooms. Higher pressures are used in clean areas, as opposed to dirty or biohazardous ones, in order to minimize contamination. Generally, a differential pressure of 2,5 mm to 5,0 mm mercury is maintained.

J.2.5 Lighting

J.2.5.1 The three characteristics of light that can influence laboratory animals are intensity, quality, and photoperiod. The lighting should provide good visibility and uniform, glare-free illumination. Light tubes, which imitate the spectrum of sunlight, are commercially available and their use is recommended.

J.2.5.2 Where natural lighting is not used, light and dark periods should be at least 6 h each per day.

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J.2.5.3 Photoperiod is probably the most influential of light characteristics on laboratory animals. It is suggested that if a change occurs in an animal's photoperiod, then no experiments should be conducted with that animal for at least a week. If a long light phase is interrupted by a shorter dark phase, there are few significant effects. However, if the reverse occurs, endogenous rhythms can be significantly skewed. This is one reason why automatic timers should control light cycles in all animal rooms. Timer function should be monitored or hooked into an alarm system. A daily cycle of 12 h dark:12 h light is usual. Additionally, any windows in an animal room should be capable of being blacked out.

J.2.6 Noise

J.2.6.1 Sudden irregular noises create more disturbances in pigs than continuous or predictable sounds. Pigs are particularly aversive to sudden, loud noises, and these shall be avoided. Housing personnel should announce themselves in a uniform manner to avoid startling the pigs.

J.2.6.2 Noise cannot be eliminated from an animal unit but care should be taken to minimize the generation of sudden extraneous audible and ultrasound noise in the vicinity of animals.

J.2.7 Vibration

J.2.7.1 Vibration stability is important for the maintenance of a constant study environment for sensitive animals. Therefore, animal holding and test rooms should be located away from areas such as a cagewash, major circulation corridors where racks are frequently in transit, mechanical rooms, and elevator shafts. Vibration studies should be performed to determine how best to achieve the maximum allowable vibration levels as determined by instruments and animals to be used in the area.

J.2.7.2 Vibration stability will be of greater concern if the animal facility is located on the upper levels of a building rather than at ground level because of structural considerations.

J.3 Animal care and health

J.3.1 General

J.3.1.1 Unless there is good husbandry, veterinary or scientific justification for individual housing, animals should be maintained in compatible sociable groups. These groups should remain stable. Pigs are social animals which depend on social contact and will show severe stress reactions if separated from their group. If individual housing is required, the animals should at least have visible contact with conspecifics.

J.3.1.2 A wide variety of pig breeds are available for research and teaching purposes. Most available breeds have been selected for rapid weight gain and thus have high-feeding needs. They also grow significantly over relatively short periods of time, which can result in housing and handling difficulties when they are housed for long periods. The use of slow-growing or miniature pigs is recommended for such long-term studies.

J.3.1.3 Pigs usually spend the bulk of the day in search of food on and under the surface of the ground. Pigs are omnivores and are highly motivated to root. As such, they readily ingest most edible materials such as insects, seeds and roughage.

J.3.1.4 Pigs respond extremely well to positive food reinforcement such as the provision of popcorn, cabbage leaves or apples. Low stress handling, which is based on guiding the animals and restraining them in slings and inspection pens, can be achieved by competent, calm and confident personnel within an environment that is designed to assist such efforts.

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J.3.1.5 Failure to provide pigs with adequate space and stimulation can create stress that can cause aggressive behaviour. Other factors which contribute to stress, such as poor handling, inadequate ventilation or unsuitable temperature, can also lead to aggressive behaviour.

J.3.1.6 Since pigs are naturally inquisitive and readily chew objects in their environment, most tail biting begins with non-aggressive chewing of tails, which then leads to persistent biting and harassment. Ear and flank biting sometimes begins when persistent, redirected sucking behaviour leads to a skin injury, which then attracts chewing and biting. Instigators should be isolated and victims shall be removed from the pen and be treated.

J.3.1.7 Amongst the many factors that can contribute to tail biting are all causes of stress and discomfort, particularly crowding and ventilation problems, certain dietary problems, especially inadequate dietary protein or salt at less than 0,25 % of the diet. Teeth clipping should not replace proper management efforts to ensure animal welfare.

J.3.2 Bedding material

J.3.2.1 With the exception of slatted floors, absorbent bedding material such as straw or wood shavings should be added to interior pens to provide a clean, comfortable and dry surface, unless approved otherwise by the AEC for specific study-related requirements. A minimum average layer thickness of 5 cm of bedding material is recommended. Heavy animals, animals kept in adverse temperature conditions, recovering animals and littering sows require thicker layered bedding. Floor drains should be covered with mesh or grids to avoid them being blocked with bedding.

J.3.2.2 Pigs should be given the opportunity to create their own toilet, sleeping and feeding areas.

J.3.2.3 Bedding may be non-nutritive, but should be non-toxic, absorbent and comfortable. Resinous wood shavings, especially cedar, are not suitable for use as laboratory animal bedding. Pine shavings should be avoided for the same reason, although they are not as toxic as cedar.

J.3.2.4 Slatted floors or cages with grates or perforated bottoms require special caution. Care should be taken that the floors are specifically designed for the breed and weight class concerned, should provide secure footing, prevent injuries and be comfortable.

J.3.3 Food and water

J.3.3.1 Potable water should be supplied to animals in sufficient quantity and be presented in a manner that an animal can use. Water receptacles should be sited to avoid fouling, while still being accessible to young piglets. Tap water might be sufficient for conventional housing facilities. Housing personnel should ensure that the height of the bunk- or trough-type feeder is suitable for the animals housed.

J.3.3.2 Where large numbers of breeding or stock animals are maintained in pens, it is important to ensure that there are sufficient feeding and watering stations to avoid undue competition.

J.3.3.3 An individual animal's nutrient requirements are affected by many factors. Young animals generally need increased amounts of many nutrients. Reproduction places many demands on female animals, and nutrient requirements are very high in gestating and lactating animals. Environmental temperature and humidity might also affect food intake and nutrient needs.

J.3.3.4 All feed should be clean, free of contaminants or pests, palatable, fresh and sufficient for the animal's needs. The selected food should be a balanced diet that provides all required nutrients.

J.3.3.5 The technique of Body Condition Scoring (BCS) should be learned by all animal attendants to assess whether or not the diet of the animals in their care is maintaining the animals in good body condition.

J.3.4 Cleaning

J.3.4.1 Routine cleaning and maintenance, and a high standard of hygiene are essential for good husbandry. Suitable and institutionally approved cleaning agents and procedures should be applied.

J.3.4.2 The facilities should be designed to support manure removal, cleaning and disinfection. This relates in particular to the drainage systems as they are prone to clogging by straw or sawdust (see J.3.2.1).

J.3.4.3 Decisions on the frequency of cleaning should be based on the housing system, type of animal, stocking densities, and the ability of ventilation systems to maintain suitable air quality.

J.3.4.4 Fly, tick and other pest populations should be regularly monitored and appropriate control measures be applied when indicated.

J.3.5 Environmental enrichment

J.3.5.1 As pigs are very active and intelligent animals, they require sufficient enrichment to avoid frustration and boredom. Piglets play with each other, or play with toys (for example, suspended ropes or balls). Pigs of all ages will readily engage with enrichment objects that return food items as reward (for example, activity feeders), suspended perforated bottles that contain popcorn or food pellets.

J.3.5.2 The widely used practice of feeding pigs twice daily is unsatisfactory from both behavioural and physiological points of view. Pigs shall either be fed *ad libitum* or food shall be provided at frequent intervals. Pigs can and will ingest roughage, such as straw, hay or sawdust, to fill their gut, even if adequate nutrients are provided in the formulated diet.

J.3.5.3 Environmental enrichment objects should be maintained in a clean condition as pigs avoid objects soiled with manure. Pigs also will often bite and chew on the objects, and care should be taken to ensure appropriate materials are used and broken objects are removed to avoid ingestion or injury (or both).

J.3.6 Animal accommodation (see table 14)

J.3.6.1 Pig housing facilities should provide suitable access and restraining devices to allow animals to be inspected, caught or moved as necessary.

J.3.6.2 If pigs are maintained over longer periods, they should be exercised regularly (for example, by walking them up and down the aisle). Such a programme not only provides some exercise and stimulation, but also facilitates moving the animals around in order to become familiar with their environment.

J.3.6.3 Pens should be of sturdy construction to contain the animals securely and should be designed and maintained to prevent pigs from becoming trapped or injuring themselves. This is of particular importance in the case of piglets when they are kept in pens designed for older age groups.

J.3.6.4 Space allowances for pigs vary greatly depending on animal size, gestation status, lactation status, climate conditions, etc. In general, pens should be large enough to allow all pigs to

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lie comfortably on a dry and bedded area. During transport or when in other pens where pigs are kept for short periods, enough space should be allowed for all animals to stand comfortably.

J.3.6.5 For specific purposes (for example, immediate post-operative care or metabolic studies) it might be justified to restrict the available space or other aspects of the primary enclosure (or both). Such studies should state these conditions clearly in the proposal to the AEC for it to be approved.

J.3.7 Breeding

J.3.7.1 Farrowing units should be designed to facilitate the safe control of the sows and yet allow unrestricted nursing of the piglets. Perimeter rails or wall cut-outs with attached creep areas are important to protect piglets from accidental crushing by sows.

J.3.7.2 The design of farrowing pens should take into consideration the often large dimensions of sows, and also allow for feeding troughs and watering points. Square pens measuring 2,4 m x 2,4 m are recommended.

J.3.7.3 Sows should be allowed to give birth with minimum interference. Animal attendants should be familiar with normal birth and should be able to recognize problems. Assistance in birthing should, if necessary, be provided under veterinary supervision.

J.3.7.4 Detailed records should be kept of pedigrees as well as of fertility and rearing success.

J.3.8 Animal identification

J.3.8.1 General

J.3.8.1.1 The most important considerations in choosing a marking technique concern its effect on the behaviour, physiology and survival of the animal. Any technique that causes an adverse effect on the animal is not only inhumane, but is likely to distort the data being collected, resulting in meaningless and often misleading results.

J.3.8.1.2 In choosing an acceptable marking technique, the researcher should consider the nature and duration of restraint, the amount of tissue removed or damaged, whether or not pain, if inflicted, is momentary or prolonged, and whether the risk of infection and abscessation is minimal.

J.3.8.2 Permanent marking

J.3.8.2.1 Ear-notching provides an acceptable manner to number and thus uniquely identify pigs. This should be carried out by an experienced operator, using properly maintained instruments and good hygienic technique.

J.3.8.2.2 Microchips are widely used to uniquely identify animals. New generation microchips even allow for the measuring of body temperature or the storage of animal data on the chip.

J.3.8.2.3 Ear-tags of a suitable size for small livestock are widely available and often used. More than two tags per ear is considered excessive. When reapplying tags, the operator should use the pre-existing hole(s) in the ear.

J.3.8.2.4 Tattoos on one or both ears may also be used. Tattooing should be carried out by an experienced operator, using properly maintained equipment and good hygienic practice.

NOTE Owing to their ease of identification and application, ear-tags have largely replaced tattoos.

J.3.8.3 Temporary marking

Pigs are often marked with marking sticks that leave a strip of colour on the skin. This is easily applied but only lasts for several days, and then it can be reapplied.

J.3.9 Handling

J.3.9.1 While pigs should be well familiarized with their handlers, they should be restrained as little as possible. This is not a contradiction *per se* as pigs can easily be moved or even handled without being restrained as such. Providing pigs with regular, positive human contact, for example, allowing the animals to approach handlers, to explore without fear, stroking them firmly and talking to them, is another way of familiarizing pigs with their handlers.

J.3.9.2 Pigs do respond extremely well to training, and can be persuaded to co-operate during common procedures such as weighing, clinical examination and even blood collection.

J.3.9.3 The facility should be designed and maintained to allow easy movement of pigs throughout their stay at a facility. This includes loading and off-loading facilities, scales, chutes and tunnels, between pens, etc. Pigs have a strong tendency to follow each other and to maintain both visual and body contact with each other. Pigs may balk at contrasting shadows, bright spots, and changes in floor surface. Pigs also have a strong escape reaction. When prodded, a pig will attempt to get away, either by running forward or by turning back to shelter amongst the group.

J.3.9.4 Chase boards (about 1 m wide) are a preferred device for moving pigs. These are usually made of plywood or aluminium with handholds. The attendant can walk behind the pigs and keep them moving in the right direction. If required, pigs should be lifted with proper support for the chest and abdomen and should never be caught, lifted or moved by their ears, tails or legs.

J.3.9.5 Recommended restraining devices include the "Panepinto Sling" (see bibliography) which is basically a hammock that has four holes cut out to accommodate the pig's legs, and the use of hog boards to separate, guide and gently restrain pigs.

J.3.10 Records

Regular monitoring of health and reproductive data, and keeping detailed records thereof, is essential to ensure that problems are identified at an early stage so that corrective action can be implemented to minimize any potentially adverse welfare effects on the animals. This form of monitoring and assessment is of particular importance in groups, where large numbers of animals are maintained, or where there is a high animal turnover.

J.4 References

See bibliography.

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Annex K

(informative)

Care and management of laboratory animals — Rabbits and guinea pigs

K.1 Introduction

K.1.1 General

Laboratory rabbits and guinea pigs are highly adaptable animals that are selected for important traits such as docility and the ability to breed in laboratory conditions. However, they do retain many of the traits of their wild counterparts, such as grooming, exploratory activity, searching for food, burrowing and gnawing, and housing systems shall aim to provide for these behavioural needs.

K.1.2 Rabbits

Wild rabbits are social animals that interact with each other whether living in large groups or in small groups. Aggression among females is limited, although dominance hierarchies are formed, and females with young will chase other rabbits away from their nests. Aggression among males increases as they approach puberty and consists mainly of chasing, with one rabbit trying to get out of the sight of the other and leading to serious injuries when this is not possible. Amicable interactions (for example, mutual grooming and lying close together) are usually seen only in the sexual context between a buck and a doe. Female-to-female amicable interactions occur under laboratory conditions in the absence of males. Young rabbits sport and play with each other and with inanimate objects. In the wild, rabbits dig burrows to hide and nest in, and they also dig for the roots of plants. In the laboratory, rabbits will dig for no obvious reason which indicates that they are highly motivated to engage in this activity. The rabbit is a naturally gregarious species, so attention should be paid to their social wellbeing.

K.1.3 Guinea pigs

Guinea pigs are domesticated, conspicuously docile, social rodents that originate from South America. The fact that they emit squeaky sounds like little pigs is the reason for their misleading name. They live in small groups of five to ten individuals. Even though they do not groom one another, guinea pigs seek each other's bodily contact during periods of rest. Guinea pigs neither compete over food nor do they hoard food. This leaves little reason for aggressive disputes. Females never engage in fighting and only rarely have harmless squabbles with each other. They get along with each other so well that they even practice communal nursing. Males are inhibited to show any kind of aggression, including threats, against females, but they fight viciously with each other in the presence of oestrus females.

K.2 The environment

K.2.1 General

Rabbits and guinea pigs choose to manipulate their own microenvironments via activities such as huddling, nest building and tunnelling. This is more important for their welfare than specifying ambient conditions within the room, therefore, the provision of suitable bedding material and places of refuge is essential.

K.2.2 Temperature

K.2.2.1 The optimal temperature range for housing rabbits is 16 °C to 22 °C, and 20 °C to 24 °C for guinea pigs. Temperature regulation should be such as to ensure that there are no undue fluctuations that could cause unnecessary stress or clinical welfare problems.

K.2.2.2 Reproductive performance can be significantly impaired if good temperature control is not maintained.

K.2.2.3 If welfare problems that can be attributed to a failure to maintain suitable temperatures occur in the animals, provision for heating or cooling (or both) will be required.

K.2.2.4 Temperatures within the cages will often be higher than room temperatures. Even with grid floors and adequate ventilation, the cage temperatures may be 3 °C to 6 °C above room temperature. The difference is likely to be greater in the solid-floored cages used for breeding. Factors affecting temperature in the cage include the type of cage and bedding or nesting material used, the use of filter covers, the age, sex, strain and species of the animal, and housing density.

K.2.2.5 Provision of bedding or nesting material allows the animal an opportunity to manipulate its own immediate environment, and provides a warm nest for its young. This might also promote greater utilization of the available space.

K.2.2.6 It is essential that emergency equipment be available to maintain environmental temperatures, particularly in rooms that house small laboratory animals.

K.2.2.7 In special cases, for example, when housing very young or hairless animals, higher room temperatures than those indicated in K.2.2.1 might be required.

K.2.2.8 Room temperature should be monitored daily, preferably by continuous recording. A less costly alternative is the use of a maximum and minimum thermometer that is examined and reset daily. However, since this does not indicate how long the room was held at a particular temperature, knowledge of which is extremely important, the use of a thermograph is therefore recommended. The temperature of the microenvironment should also be monitored.

K.2.2.9 Occasionally, optimal temperature for the laboratory animal is not the most comfortable for personnel. However, human preferences should not compromise the study requirements or the health and comfort of the animal.

K.2.3 Relative humidity

Humidity control is an important consideration for laboratory animals since it contributes to the variability of research models. For rabbits and guinea pigs, a relative humidity in the range of 55 % ± 10 % is recommended.

K.2.4 Ventilation

K.2.4.1 To maintain suitable air quality, airflow rate requirements might differ depending on the type of accommodation, with tiered racks of cages likely to require higher rates than single-tiered open mesh cages or floor pens.

K.2.4.2 As rabbits shed considerable amounts of hair, the extract ducts should be cleaned regularly to ensure continued efficiency of ventilation.

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K.2.4.3 Ventilation influences temperature, humidity, and gaseous and particulate contaminants in the animal cage and holding room. The design of the building ventilation system should permit the maintenance of these parameters within acceptable limits.

K.2.4.4 The actual ventilation rate required varies with age, sex, species, stocking density, frequency of cleaning, quality of incoming air, ambient temperature and humidity, and the type of construction of primary and secondary enclosures, among other factors.

K.2.4.5 Draft-free air exchanges in the range of 15 exchanges to 20 exchanges per hour at cage level are commonly recommended for rooms that contain small laboratory animals under conventional housing conditions.

K.2.4.6 Differential pressures can be used to inhibit the passage of pathogenic material between rooms. Higher pressures are used in clean areas, as opposed to dirty or biohazardous ones, in order to minimize contamination. Generally, a differential pressure of 2,5 mm to 5,0 mm mercury is maintained.

K.2.5 Lighting

K.2.5.1 The three characteristics of light that can influence laboratory animals are intensity, quality, and photoperiod. The lighting should provide good visibility and uniform, glare-free illumination. Lighting should be such that animals can be easily inspected. In a tier racking system, care should be taken to ensure that animals in the top tier are not exposed directly to high intensity lighting. Light tubes, which imitate the spectrum of sunlight, are commercially available and their use is recommended.

K.2.5.2 Previous recommendations of 807 lux to 1345 lux at 76 cm above the floor have been shown to cause retinal degeneration in albino rats. The recommended level of 323 lux approximately 1,0 m above the floor has proved sufficient for the performance of routine animal care duties and does not cause rodent phototoxic retinopathy. A level of approximately 200 lux does not appear to cause retinal damage and has been shown to be adequate for reproduction and normal social behaviour in most rodents. At this level, an additional light source on a separate switch is needed to enhance illumination during care-taking activities. This recommendation for albino rats can also be considered suitable for albino rabbits and albino guinea pigs.

K.2.5.3 Animals, especially when breeding, should be given the opportunity to withdraw to shaded areas within the cage (for example, via the provision of adequate nesting materials).

K.2.5.4 Photoperiod is probably the most influential of light characteristics on laboratory animals. It is suggested that if a change occurs in an animal's photoperiod, then no experiments should be conducted with that animal for at least a week. If a long light phase is interrupted by a shorter dark phase, there are few significant effects. However, if the reverse occurs, endogenous rhythms can be significantly skewed. This is one reason why automatic timers should control light cycles in all animal rooms. Timer function should be monitored or hooked into an alarm system. A daily cycle of 12 h dark:12 h light is usual. Additionally, any windows in an animal room should be capable of being blacked out.

K.2.6 Noise

K.2.6.1 Rabbits and guinea pigs are easily frightened by sudden, unexpected loud noise and might injure themselves in panic. Some forms of low-level background noise in the animal room might be beneficial in reducing the impact of sudden loud noises. Since rabbits and guinea pigs are sensitive to ultrasound, care should be taken to minimize the generation of extraneous audible and ultrasound noise in the vicinity of the animals.

K.2.6.2 Sudden, irregular noises create more disturbances in rabbits and guinea pigs than continuous or predictable sounds.

K.2.7 Vibration

K.2.7.1 Vibration stability is important for the maintenance of a constant study environment for sensitive animals. Therefore, animal holding and test rooms should be located away from areas such as a cagewash, major circulation corridors where racks are frequently in transit, mechanical rooms and elevator shafts. Vibration studies should be performed to determine how best to achieve the maximum allowable vibration levels as determined by instruments and animals to be used in the area.

K.2.7.2 Vibration stability will be of greater concern if the animal facility is located on the upper levels of a building rather than at ground level because of structural considerations.

K.3 Animal care and health

K.3.1 General

Unless there is good husbandry, veterinary or scientific justification for individual housing, animals should be maintained in compatible sociable groups. These groups should remain stable. Frequent mixing of groups of breeding rabbits and guinea pigs is strongly discouraged since this can be a source of intense stressful conflict.

K.3.2 Bedding and nesting material

K.3.2.1 Cages may use direct bedding (the animals are in direct contact with the bedding) or indirect bedding (animals are on a grate above the bedding or perforated bottom cages).

K.3.2.2 Nesting materials are crucial to breeding rabbits to enable them to engineer appropriate microenvironments that facilitate the successful rearing of the young. Unlike other rodents, guinea pigs do not construct nests. However, bedding of dust-free shavings supplemented daily with high-quality hay should be regarded as a basic form of environmental and feeding enrichment.

K.3.2.3 Bedding may be non-nutritive, but should be non-toxic, absorbent and comfortable. Dust-free wood shavings, corncob and straw are typical beddings. Resinous wood shavings, especially cedar, are not suitable for use as laboratory animal bedding. Pine shavings should be avoided for the same reason, although they are not as toxic as cedar.

K.3.3 Food and water

K.3.3.1 Guinea pigs are unable to synthesize vitamin C (ascorbic acid) in sufficient quantity to meet their daily requirements. It is therefore essential that their diet be of suitable composition to meet this requirement.

K.3.3.2 Rabbits and guinea pigs need to engage in regular gnawing behaviour to prevent overgrowth of their front teeth. Hard food pellets, carrots and softwood sticks are suitable to meet this need.

K.3.3.3 Potable water should be supplied to animals in sufficient quantity and be presented in a manner that an animal can use. Tap water might be sufficient for conventional housing facilities, but for specific pathogen-free (SPF) or barrier units, water should be sterilized. Water sterilization is

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easily achieved by autoclaving the filled water bottles or by acidifying water supplies to a pH value of 2,5. This procedure should be carefully controlled and taken into account as a study variable.

K.3.3.4 Where large numbers of breeding or stock animals are maintained in a single cage or pen, it is important to ensure that there are sufficient feeding and watering stations to avoid undue competition.

K.3.3.5 An individual animal's nutrient requirements are affected by many factors. Young animals generally need increased amounts of many nutrients. Reproduction places many demands on female animals, and nutrient requirements are very high in gestating and lactating animals. Environmental temperature and humidity can also affect food intake and nutrient needs.

K.3.3.6 Most rabbits and guinea pigs are fed a combination of standardized diet and lucerne or hay (or both). All feed should be clean, free of contaminants or pests, palatable, fresh and sufficient for the animal's needs. The selected food should be a balanced diet that provides all required nutrients.

K.3.3.7 For SPF or barrier units, feed should be sterilized either by autoclaving at a low temperature (resulting in nutrient loss) or by radiation.

K.3.4 Cleaning

K.3.4.1 Routine cleaning and maintenance, and a high standard of hygiene are essential for good husbandry. Suitable or institutionally approved cleaning agents and procedures should be applied.

K.3.4.2 Decisions on the frequency of cleaning should be based on the housing system, type of animal, stocking densities, and the ability of ventilation systems to maintain suitable air quality.

K.3.5 Environmental enrichment

K.3.5.1 The welfare of rabbits housed in cages may be enhanced by environmental enrichment, (for example, through the provision of hay, hay blocks or chew sticks).

K.3.5.2 Enrichment in floor pen systems is readily achieved by, for example, the incorporation of different compartments within a pen and the use of boxes or pipes for concealment. The use of straw for bedding and hay in the diet provides additional environmental enrichment. Post-weaned animals should be maintained for as long as is possible, in compatible groups.

K.3.5.3 Guinea pigs are social animals and should therefore be maintained in groups or in breeding pairs. Single housing should only be used if there is good veterinary or husbandry justification.

K.3.5.4 Although nesting material is not an essential requirement for non-breeding guinea pigs, some form of bedding material should be provided. The use of hay or a similar substrate will increase environmental complexity in a sterile cage environment, will encourage better utilization of the available space, and will provide the opportunity for concealment. The addition of sterilized soft wood sticks for guinea pigs to gnaw may also be considered.

K.3.6 Animal accommodation (see tables 15 and 16)

K.3.6.1 Solid-floored cages, floor pens or mesh-floored cages are used for the house breeding of stock rabbits and guinea pigs. These cages can be suspended, tiered on racks or mounted on bases.

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K.3.6.2 With competent management and good husbandry practices, there are welfare benefits to be gained from animals housed in social groups in floor pen accommodation, where a wider behavioural and locomotor repertoire can be expressed.

K.3.6.3 Suspended mesh caging offers advantages over conventional racked solid caging, i.e. the animals have good visual field, and have some social contact with adjacent rabbits. Animals are more easily observed, but one disadvantage is the lack of concealment. A suspended wire cage system has the added benefit in that good air quality and movement might be more easily maintained.

K.3.6.4 Guinea pigs need the social environment to guarantee their behavioural health and to safeguard their physiological wellbeing.

K.3.6.5 Although mesh-floored cages might offer some advantages over solid floor cages (for example, reducing disturbance during cleaning and eliminating cage flooding with automatic watering systems), it is essential that they are suitable for heavy rabbits and guinea pigs. Heavy animals are prone to developing pressure sores and pododermatitis on wire mesh floors.

The mesh should be carefully inspected and well maintained to ensure that there are no loose or sharp projections. Prompt action should be taken to correct all faults found or to replace the mesh floors with a solid-bottomed cage. Faulty mesh floors can lead to serious injuries.

K.3.6.6 A suitable substrate should be provided. Hay is frequently used for this purpose. In addition to the nutritional value to the animal, it provides a form of environmental enrichment. When hay is not used, reproductive performance might be reduced and an increase in stereotypic behaviour seen.

K.3.6.7 Study and care-planning should be aimed at allowing the group housing of social animal species.

K.3.7 Breeding

K.3.7.1 Nesting boxes should be provided for breeding does. Some substrate, for example, hay or shredded paper, should be provided as bedding material. The box should be available for several days before littering to permit the doe to exhibit normal nesting behaviour.

K.3.7.2 The nesting area should be designed to contain the young rabbits in the early post-partum period, but should be of sufficient size to permit suckling.

K.3.7.3 The young rabbits emerge from the nesting box at two weeks to three weeks of age and are generally weaned at six weeks. Wherever possible, littermates should be housed in groups post-weaning. This facilitates subsequent group-housing programmes.

K.3.7.4 Does should be assessed for continued suitability for breeding before mating.

K.3.7.5 Guinea pigs are generally bred as breeding pairs or in harems. The offspring are fully developed at birth. Weaning takes place at two weeks to three weeks, but the young generally eat solid food and drink water within a few days of birth. Young animals should be maintained in compatible groups.

K.3.7.6 Disturbance of the animals should be minimized during late pregnancy and early lactation to reduce the risk of mismothering or cannibalism.

K.3.7.7 Detailed records should be kept of pedigrees as well as of fertility and rearing success.

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K.3.8 Animal identification

K.3.8.1 General

K.3.8.1.1 The most important considerations in choosing a marking technique concern its effect on the behaviour, physiology and survival of the animal. Any technique that causes an adverse effect on the animal is not only inhumane, but is likely to distort the data being collected, resulting in meaningless and often misleading results.

K.3.8.1.2 In choosing an acceptable marking technique, the researcher should consider the nature and duration of restraint, the amount of tissue removed or damaged, whether or not pain, if inflicted, is momentary or prolonged, and whether the risk of infection and abscessation is minimal.

K.3.8.2 Permanent marking

K.3.8.2.1 Ear clipping provides an acceptable manner to number and thus uniquely identify guinea pigs.

K.3.8.2.2 Microchips are widely used to uniquely identify animals. New generation microchips even allow for the measuring of body temperature or the storage of animal data on the chip. Due to the large gauge of the implanting needle, the implantation of microchips in rabbits and guinea pigs should always be performed under general anaesthesia in sterile conditions.

K.3.8.3 Semi-permanent marking

A patch of fur or patterns on the back or side of the animal may be shaved, clipped or cut with a pair of scissors. Such marks generally last one week to four weeks (depending on the stage of the hair cycle) and can be used on any colour animal.

K.3.8.4 Temporary marking

A felt tip marker may be used for marking an ear or tail. This is easily applied but only lasts for 1 d to 2 d, and then it can be reapplied. Food colouring may be used to dye a patch of fur. Such marks generally last for one week to two weeks, but can be used only on albino and light-coloured animals. In dark-coloured animals, hair can be bleached with peroxide or commercial hair treatment products. However, such procedures require extreme caution to avoid skin damage, accidental ingestion or damage to eyes and other structures, and are best applied under anaesthesia.

K.3.9 Handling

K.3.9.1 Group-housed rabbits should be caught with minimum chasing. One can make use of the rabbit's natural tendency to hide when startled (for example, under a resting board or box), where they can be identified, picked up and handled in a gentle and skilful manner. Any dark hiding place will serve the same purpose, but a quiet, smooth approach is required. It is important not to startle the animal in its hiding place. Once the animals are used to being picked up, they might not even hide from a technician they know well. The anticipation of what is to happen after being caught plays a major role in the rabbit's behaviour. Procedures carried out with rabbits should be as free of stress as possible. Rabbits who are used to being treated with compassion and professional skill will not panic in anticipation of procedures. Carefully bundling a rabbit in a blanket and gently covering his or her eyes with a towel usually has a calming effect, even on a very agitated animal.

K.3.9.2 Rabbits and guinea pigs should be handled in such a way as to minimize any injuries. Rabbits should always be given support in the pelvic region to prevent broken backs and, guinea pigs should always be handled with both hands to prevent broken ribs.

K.3.9.3 Blood sampling is least stressful if the subject is given a sedative and an analgesic. The added advantage is that the arteries and veins are dilated, making it easier to take the samples.

Local anaesthetics creams that contain, for example, lidocaine and prilocaine at 2,5 % each, might serve the same purpose.

K.3.9.4 To a considerable extent, proper handling depends on the handler rather than on the animal subject.

K.3.10 Records

K.3.10.1 Each rabbit and guinea pig should be individually identifiable and individual records shall be kept.

K.3.10.2 Regular monitoring of health and reproductive data, and keeping detailed records thereof, is essential to ensure that problems are identified at an early stage so that corrective action can be implemented to minimize any potentially adverse welfare effects on the animals. This form of monitoring and assessment is of particular importance in groups, where large numbers of animals are maintained in breeding colonies, or where there is a high animal turnover.

K.3.11 References

See bibliography.

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Annex L

(informative)

Care and management of laboratory animals — Rodents (mice, rats and hamsters)

L.1 Introduction

L.1.1 General

Laboratory rodents are highly adaptable animals that are selected for important traits such as docility and the ability to breed in laboratory conditions. However, they do retain many of the traits of their wild counterparts, such as grooming, exploratory activity, searching for food, burrowing and gnawing, and housing systems shall aim to encompass these behavioural needs.

L.1.2 Mouse

The laboratory mouse is derived from a largely nocturnal burrowing and climbing ancestor, which favoured building nests for temperature regulation and reproduction. Mice do not readily cross open spaces, as confirmed by the use of cage space studies. Mice are capable of assuming a wide range of social organizations and intense territoriality might be seen in reproductively active males. Pregnant and lactating females might prove aggressive in nest defence. As mice have poor sight, particularly the albino strains, they rely heavily on the sense of smell and create patterns of urine markings in their environment.

L.1.3 Rat

As the rat is a very much more of a social animal than the mouse, disruption to social groups should be minimized. Young animals are very exploratory and interact to an enormous degree. Rats are excellent climbers, avoid open spaces, and use urine spotting as a territorial marker. The senses of smell and hearing are highly developed, and these animals are particularly sensitive to ultrasound. Daylight vision is poor, but dim-light vision is effective in some pigmented strains. Activity is higher during hours of darkness.

L.1.4 Hamster

The hamster species is very different from the mouse and the rat. The female is larger and more aggressive than the male. During pregnancy and lactation, the female can be intensely aggressive and can inflict serious injury on her mate. Male hamsters can be group-housed successfully if this is introduced from weaning age. Female hamsters should be individually housed. Female hamsters often provide a latrine area within the cage, mark areas with secretions from a flank gland, and frequently selectively reduce the size of their own litter by cannibalism. Careful control of environmental features, and prevention of disruption during routine husbandry practices are of particular importance in this species.

L.2 The environment

L.2.1 General

Laboratory rodents are species that choose to manipulate their own microenvironments via activities such as huddling, nest building and tunnelling. In general, the rodent's ability to control temperature, humidity and lighting is more important to its welfare than specifying ambient conditions within the room. The microclimate within the cage is of most importance to the animal,

and welfare seems facilitated when rodents are able to control this (for example, via the provision of bedding material).

Microenvironment is especially important for hamsters since it determines whether the animals go into a state of hibernation or not. Constant temperature and lighting are necessary to prevent this. Care should be taken not to mistake hibernating hamsters as ill or dead.

L.2.2 Temperature

L.2.2.1 The optimal temperature range for mice, rats and hamsters is 20 °C to 24 °C.

L.2.2.2 Temperatures within the cages will often be higher than room temperatures. Even with grid floors and adequate ventilation, the cage temperatures may be 3 °C to 6 °C above room temperature. The difference is likely to be greater in the solid-floored cages used for breeding. Factors affecting temperature in the cage include the type of cage and bedding or nesting material used, the use of filter covers, the age, sex, strain and species of the animal, and housing density.

L.2.2.3 Provision of bedding or nesting material allows the animal an opportunity to manipulate its own immediate environment, and provides a warm nest for its young. This might also promote greater utilization of the available space.

L.2.2.4 It is essential that emergency equipment be available to maintain environmental temperatures, particularly in rooms housing small laboratory animals.

L.2.2.5 In special cases, for example, when housing very young or hairless animals, higher room temperatures than those indicated in L.2.2.1 might be required.

L.2.2.6 Room temperature should be monitored daily, preferably by continuous recording. A less costly alternative is the use of a maximum and minimum thermometer that is examined and reset daily. However, since this does not indicate how long the room was held at a particular temperature, knowledge of which is extremely important, the use of a thermograph is therefore recommended. The temperature of the microenvironment should also be monitored.

L.2.2.7 Occasionally, optimal temperature for the laboratory animal is not the most comfortable for personnel. However, human preferences should not compromise the study requirements or the health and comfort of the animal.

L.2.3 Relative humidity

L.2.3.1 Humidity control is an important consideration for laboratory animals since it contributes to the variability of research models. For rodents, a relative humidity in the range of 55 % ± 15 % is acceptable. Most laboratory animals prefer a relative humidity of approximately 60 %, but can tolerate a range of 40 % to 70 % as long as it remains relatively constant and the temperature range is appropriate.

L.2.3.2 Since a low relative humidity might contribute to the development of ringtail in rats, levels of less than 40 % shall be avoided.

L.2.4 Ventilation

L.2.4.1 Ventilation influences temperature, humidity, and gaseous and particulate contaminants in the animal cage and holding room. The design of the building ventilation system should permit the maintenance of these parameters within acceptable limits.

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L.2.4.2 The actual ventilation rate required varies with age, sex, species, stocking density, frequency of cleaning, quality of incoming air, ambient temperature and humidity, and the type of construction of primary and secondary enclosures, among other factors.

L.2.4.3 Draft-free air exchanges in the range of 15 exchanges to 20 exchanges per hour at cage level are commonly recommended for rooms that contain small laboratory animals under conventional housing conditions. Achieving these rates does not guarantee adequate ventilation at the cage level, particularly if filter-tops are used.

L.2.4.4 Laminar flow units and rooms provide good ventilation with a unidirectional airflow with few eddy currents. These systems might effectively isolate cages thus controlling the spread of odours and airborne pathogens.

L.2.4.5 Differential pressures can be used to inhibit the passage of pathogenic material between rooms. Higher pressures are used in clean areas, as opposed to dirty or biohazardous ones, in order to minimize contamination. Generally, a differential pressure of 2,5 mm to 5,0 mm mercury is maintained.

L.2.5 Lighting

L.2.5.1 The three characteristics of light that can influence laboratory animals are intensity, quality, and photoperiod. The lighting should provide good visibility and uniform, glare-free illumination. Light tubes, which imitate the spectrum of sunlight, are commercially available and their use is recommended.

L.2.5.2 Previous recommendations of 807 lux to 1345 lux at 76 cm above the floor have been shown to cause retinal degeneration in albino rats. The recommended level of 323 lux approximately 1,0 m above the floor has proved sufficient for the performance of routine animal care duties and does not cause rodent phototoxic retinopathy. A level of approximately 200 lux does not appear to cause retinal damage and has been shown to be adequate for reproduction and normal social behaviour in most rodents. At this level, an additional light source on a separate switch is needed to enhance illumination during care-taking activities.

L.2.5.3 Light levels within cages are more important to the welfare of breeding rodents than the light level in the room. Lighting intensity should be only that which is required by husbandry practices or safety reasons.

L.2.5.4 The intensity experienced by animals housed close to the source might differ markedly from that experienced by those farther away because light intensity is inversely proportional to the square of the distance from its source. Additionally, light intensity within a cage is dependent upon cage type and construction, position of the cage on the rack, and type of rack, and might vary markedly from the front to the back of a cage. Light intensity can influence aggressiveness and the incidence of cannibalism in rodents. Gradual changes between dark and light periods allow time for behavioural adjustment and the expression of crepuscular behaviour.

L.2.5.5 More use should be made of subdued lighting (for example, red lighting which rodents cannot detect). All racks (especially those that are relatively high) should have shaded tops to prevent animals in the top row being exposed to excessive light (which can cause retinal degeneration). Animals, especially when breeding, should be given the opportunity to withdraw to shaded areas within the cage (for example, via the provision of adequate nesting materials).

L.2.5.6 Photoperiod is probably the most influential of light characteristics on laboratory animals. It is suggested that if a change occurs in an animal's photoperiod, then no experiments should be conducted with that animal for at least a week. If a long light phase is interrupted by a shorter dark phase, there are few significant effects. However, if the reverse occurs, endogenous rhythms can

be significantly skewed. This is one reason why automatic timers should control light cycles in all animal rooms. Timer function should be monitored or hooked into an alarm system. A daily cycle of 12 h dark:12 h light is usual. Additionally, any windows in an animal room should be capable of being blacked out.

L.2.6 Noise

L.2.6.1 Sudden, irregular noises create more disturbances in breeding rodents than continuous or predictable sounds.

L.2.6.2 Since rodent neonates use ultrasound production to communicate distress, it is important that extraneous noise be minimized. Ultrasound from cleaning devices, pressure hoses, trolley wheels, vacuum cleaners, computer visual display units (VDUs) might result in abnormal behaviour and disturbed breeding cycles.

L.2.6.3 Noise cannot be eliminated from an animal unit but care should be taken to minimize the generation of sudden extraneous audible and ultrasound noise in the vicinity of animals.

L.2.7 Vibration

L.2.7.1 Vibration stability is important for the maintenance of a constant study environment for sensitive animals such as rodents. Therefore, rodent holding and test rooms should be located away from areas such as a cagewash, major circulation corridors where racks are frequently in transit, mechanical rooms and elevator shafts. Vibration studies should be performed to determine how best to achieve the maximum allowable vibration levels as determined by instruments and animals to be used in the area.

L.2.7.2 Vibration stability will be of greater concern if the animal facility is located on the upper levels of a building rather than at ground level because of structural considerations.

L.3 Animal care and health

L.3.1 General

Unless there is good husbandry, veterinary or scientific justification for individual housing, animals should be maintained in compatible sociable groups. These groups should remain stable. Frequent mixing of groups of breeding rodents is strongly discouraged since this can be a source of intense stressful conflict.

L.3.2 Bedding and nesting material

L.3.2.1 Cages may use direct bedding (the animals are in direct contact with the bedding) or indirect bedding (animals are on a grate above the bedding or perforated bottom cages).

L.3.2.2 Nesting materials are crucial to breeding rodents to enable them to engineer appropriate microenvironments that facilitate the successful rearing of the young. The bedding is also an important material on which all three species lay down patterns of odour cues which are important for the animal's sense of security.

L.3.2.3 Bedding may be non-nutritive, but should be non-toxic, absorbent and comfortable. Wood shavings, corncob and vermiculite are typical beddings. Resinous wood shavings, especially cedar, are not suitable for use as laboratory animal bedding. Pine shavings should be avoided for the same reason, although they are not as toxic as cedar.

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L.3.2.4 Caution should be used with animals housed in cages with grates or perforated bottoms.

L.3.3 Food and water

L.3.3.1 Potable water should be supplied to animals in sufficient quantity and be presented in a manner that an animal can use. Tap water might be sufficient for conventional housing facilities, but for SPF or barrier units, water should be sterilized. Water sterilization is easily achieved by autoclaving the filled water bottles or by acidifying water supplies to a pH value of 2,5. This procedure should be carefully controlled and taken into account as a study variable. Mice might adapt to the change from tap to acidified water through time.

L.3.3.2 Where large numbers of breeding or stock animals are maintained in a single cage or pen, it is important to ensure that there are sufficient feeding and watering stations to avoid undue competition.

L.3.3.3 An individual animal's nutrient requirements are affected by many factors. Young animals generally need increased amounts of many nutrients. Reproduction places many demands on female animals, and nutrient requirements are very high in gestating and lactating animals. Environmental temperature and humidity can also affect food intake and nutrient needs.

L.3.3.4 Most rodents are fed a standardized diet. All feed should be clean, free of contaminants or pests, palatable, fresh and sufficient for the animal's needs. The selected food should be a balanced diet that provides all required nutrients.

L.3.3.5 For SPF or barrier units, feed should be sterilized either by autoclaving at a low temperature (resulting in nutrient loss) or by radiation.

L.3.3.6 Since rodents' teeth continue to grow during their entire life, the teeth need to be worn down by chewing to prevent overgrowth. Hard food pellets are usually sufficient to prevent this, but the addition of chewing sticks is recommended as part of the enrichment programme.

L.3.4 Cleaning

L.3.4.1 Routine cleaning and maintenance, and a high standard of hygiene are essential for good husbandry. Suitable and institutionally approved cleaning agents and procedures should be applied.

L.3.4.2 There is, however, a real danger of over-cleaning cages used by pregnant animals and females with litters. Such disturbances can result in mismothering or cannibalism.

L.3.4.3 Odour marking is an important activity in these rodent species and cleaning disturbances will cause a degree of social disruption. Partial cleaning (for example, removal and replacement of soiled bedding) permits some odour cues to remain in the cage and reduces the disturbance to the animals.

L.3.4.4 Decisions on the frequency of cleaning should be based on the housing system, type of animal, stocking densities, and the ability of ventilation systems to maintain suitable air quality.

L.3.5 Environmental enrichment

L.3.5.1 Many rodent species attempt to divide up their own cages into areas for feeding, resting, urination and food storage. These divisions might be based on odour marks rather than physical division, but partial barriers might be beneficial. To increase environmental complexity, the addition of some form of cage enrichment is strongly recommended. Corrugated devices or tubes are

examples of devices that have been used successfully for rodents and these have the added benefit of increasing floor utilization.

L.3.5.2 Since these rodent species are generally social animals, disruption of established groups shall be minimized as this can be very stressful.

L.3.6 Animal accommodation (see tables 17 and 18)

L.3.6.1 Cage enrichment and social interaction are considered to be of more value to the animal than simple floor space allocation. Indeed large featureless cages can induce anxiety in rats.

L.3.6.2 Young animals should be maintained in compatible groups.

L.3.6.3 Adult male mice, particularly C57/Bl6 mice, tend to become aggressive even when weaned together and should be housed individually. It might also become necessary to house other animals individually for a number of reasons such as the requirements of the study or for health concerns. However, study and care-planning should be aimed at allowing the group housing of social animal species.

L.3.7 Breeding

L.3.7.1 Rodents should be bred on solid floors and be provided with suitable bedding material, such as shredded paper or wood chippings, from which a nest can be constructed. This is important in the thermoregulation of the microenvironment, and keeps the young together for efficient lactation.

L.3.7.2 Disturbance to the animals should be minimized during late pregnancy and early lactation to reduce the risk of mismothering or cannibalism.

L.3.7.3 Detailed records should be kept of pedigrees as well as of fertility and rearing success.

L.3.8 Animal identification

L.3.8.1 General

L.3.8.1.1 The most important considerations in choosing a marking technique concern its effect on the behaviour, physiology and survival of the animal. Any technique that causes an adverse effect on the animal is not only inhumane, but is likely to distort the data being collected, resulting in meaningless and often misleading results.

L.3.8.1.2 In choosing an acceptable marking technique, the researcher should consider the nature and duration of restraint, the amount of tissue removed or damaged, whether or not pain, if inflicted, is momentary or prolonged, and whether the risk of infection and abscessation is minimal.

L.3.8.2 Permanent marking

L.3.8.2.1 Toe, ear and tail clipping

Toe, ear and tail clipping provide an acceptable manner to number and thus uniquely identify rodents, particularly mice. Should genetic monitoring be required, material retrieved by ear clipping should preferably be used for DNA extraction.

When toe clipping or tail docking are felt to be the only methods that can meet the requirements of a particular study, their use shall be reviewed and approved by the institutional AEC before implementation. Where toe clipping is used, no more than one toe per foot should be removed.

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L.3.8.2.2 Microchips

Microchips are widely used to uniquely identify animals. New generation microchips even allow for the measuring of body temperature or the storage of animal data on the chip. Due to the large gauge of the implanting needle, the implantation of microchips in rodents should always be performed under general anaesthesia in sterile conditions.

L.3.8.3 Semi-permanent marking

A patch of fur or patterns on the back or side of the rodent may be shaved, clipped or cut with a pair of scissors. Such marks generally last from one week to four weeks (depending on the stage of the hair cycle) and can be used on any colour rodent.

L.3.8.4 Temporary marking

A felt-tip marker may be used for marking an ear or tail. This is easily applied but only lasts for 1 d to 2 d, and then it can be reapplied. Food colouring may be used to dye a patch of fur. Such marks generally last for one week to two weeks, but can be used only on albino and light-coloured rodents. In dark-coloured rodents, hair can be bleached with peroxide or commercial hair treatment products. However, such procedures require extreme caution to avoid skin damage, accidental ingestion or damage to eyes and other structures, and are best applied under anaesthesia.

L.3.9 Handling

L.3.9.1 Rats can lose their shyness of people if a little time is spent handling them as juveniles. Gentle handling during infancy makes rats less fearful and quasi-tame in situations in which control rats remain timidly crouched at the back of the cage.

L.3.9.2 Rats are usually handled well by being picked up with a firm-and-gentle hold over the shoulders and quickly supported by allowing their feet to rest on the other hand or sleeve. To a considerable extent, proper handling depends on the handler rather than on the animal subject.

L.3.9.3 Laboratory mice are easily handled if approached correctly. They should be picked up by the base of the tail (never by its tip) for placement on a surface, which they can grip with their toes. They should then immediately be grasped with thumb and forefinger, by the loose skin at the base of the neck, lifted up and their tail placed between the little finger and palm, or between the fourth and fifth fingers. If forceps are used to lift the mouse out of its box, these should be rubber tipped.

L.3.9.4 When manipulations and treatments are necessary that do not involve pain, the animal can usually be picked up and restrained manually and without any difficulty.

NOTE Hamsters are solitary animals, with a tendency at certain times to be rather aggressive towards each other. However, they probably do not deserve their reputation for ill temper and biting; in fact, they tend to be naturally inquisitive and friendly.

L.3.9.5 Hamsters may be picked up using cupped hands if they are docile and used to being handled, or by grasping as much of the loose skin as possible over the neck and shoulder region if they are not used to being handled. It should not be necessary to use gloves when handling these animals as it is difficult to handle an animal gently and avoid hurting it when wearing gloves. Once an animal associates a gloved hand with being hurt, it will automatically attempt to bite. The warmth of a bare hand tends to calm and relax the animal.

L.3.9.6 All movements when approaching the animal should be deliberate and not sudden. Hamsters are sound sleepers and can occasionally even be picked up without awakening, however, this is not advisable, as sudden awakening during the process will startle the animal and lead to its biting the handler. It is therefore advisable to wake up a hamster before attempting to pick it up.

L.3.10 Records

Regular monitoring of health and reproductive data, and keeping detailed records thereof, is essential to ensure that problems are identified at an early stage so that corrective action can be implemented to minimize any potentially adverse welfare effects on the animals. This form of monitoring and assessment is of particular importance in rodent units, or where large numbers of animals are maintained in breeding colonies, or where there is a high animal turnover.

L.4 References

See bibliography.

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Annex M

(informative)

Care and management of laboratory animals — Sheep and goats

M.1 General

Found throughout the world, sheep and goats are raised in a wide variety of situations and adapted to a broad range of environmental conditions. Their ability to thrive on adverse rations, their adaptability and their unique meat, fibre and digestible milk have added to the quality of life of many people. It is important to understand their needs and the basic conditions that are necessary for sheep and goats to thrive.

M.2 The environment

M.2.1 General (outdoors)

M.2.1.1 Sheep and goats can be acclimatized to adverse climatical conditions. For reasons of providing standardized research environments, these animals are often stabled in environmentally-controlled facilities.

M.2.1.2 If sheep and goats are housed outdoors, they require proper shelter from the sun, wind, rain and other adverse weather conditions. They also require access to a dry, well-drained area for rest and rumination. This area should be large enough to accommodate all sheep and goats lying down at the same time.

M.2.2 Temperature (indoors)

M.2.2.1 Sheep and goats housed indoors should generally be maintained at room temperatures between 16 °C and 22 °C.

M.2.2.2 In special cases, for example, when housing very young or recovering animals, higher room temperatures than those indicated (see M.2.2.1) might be required. Gradual acclimatization should be done before moving them outdoors after they have either been sheared or adapted to indoor conditions.

M.2.2.3 Room temperature should be monitored daily, preferably by continuous recording. A less costly alternative is the use of a maximum and minimum thermometer that is examined and reset daily. However, since this does not indicate how long the room was held at a particular temperature, knowledge of which is extremely important, the use of a thermograph is therefore recommended. The temperature of the microenvironment should also be monitored.

M.2.2.4 Occasionally, optimal temperature for the laboratory animal is not the most comfortable for personnel. However, human preferences should not compromise the study requirements or the health and comfort of the animal.

M.2.3 Relative humidity

Humidity control is an important consideration for laboratory animals since it contributes to the variability of research models. For sheep and goats, a relative humidity in the range of 55 % ± 15 % is acceptable. Most animals prefer a relative humidity around 60 %, but can tolerate a range of 40 % to 70 % as long as it remains relatively constant and the temperature range is appropriate.

M.2.4 Ventilation

M.2.4.1 Ventilation influences temperature, humidity, and gaseous and particulate contaminants in the animal cage and holding room. The design of the building ventilation system should permit the maintenance of these parameters within acceptable limits.

M.2.4.2 The actual ventilation rate required varies with age, sex, species, stocking density, frequency of cleaning, quality of incoming air, ambient temperature and humidity, and the type of construction of primary and secondary enclosures, among other factors.

M.2.4.3 Draft-free air exchanges in the range of 10 exchanges to 15 exchanges per hour at animal level are commonly recommended for rooms that contain small livestock under conventional housing conditions.

M.2.4.4 Differential pressures can be used to inhibit the passage of pathogenic material between rooms. Higher pressures are used in clean areas, as opposed to dirty or biohazardous ones, in order to minimize contamination. Generally, a differential pressure of 2,5 mm to 5,0 mm mercury is maintained.

M.2.5 Lighting

M.2.5.1 The three characteristics of light that can influence laboratory animals are intensity, quality and photoperiod. The lighting should provide good visibility and uniform, glare-free illumination. Light tubes, which imitate the spectrum of sunlight, are commercially available and their use is recommended.

M.2.5.2 Where natural lighting is not used, light and dark periods shall be at least 6 h each per day.

M.2.5.3 Photoperiod is probably the most influential of light characteristics on laboratory animals. It is suggested that if a change occurs in an animal's photoperiod, then no experiments should be conducted with that animal for at least a week. If a longer light phase is interrupted by a shorter dark phase, there are few significant effects. However, if the reverse occurs, endogenous rhythms can be significantly skewed. This is one reason why automatic timers should control light cycles in all animal rooms. Timer function should be monitored or hooked into an alarm system. A daily cycle of 12 h dark:12 h light is usual. Additionally, any windows in an animal room should be capable of being blacked out.

M.2.6 Noise

M.2.6.1 Sudden irregular noises create more disturbances in sheep and goats than continuous or predictable sounds. Sheep are particularly fearful and sudden noises should be avoided.

M.2.6.2 Noise cannot be eliminated from an animal unit but care should be taken to minimize the generation of sudden extraneous audible and ultrasound noise in the vicinity of animals.

M.2.7 Vibration

M.2.7.1 Vibration stability is important for the maintenance of a constant study environment for sensitive animals. Therefore, animal holding and test rooms should be located away from areas such as a cagewash, major circulation corridors where racks are frequently in transit, mechanical rooms, and elevator shafts. Vibration studies should be performed to determine how best to achieve the maximum allowable vibration levels as determined by instruments and animals to be used in the area.

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M.2.7.2 Vibration stability will be of greater concern if the animal facility is located on the upper levels of a building rather than at ground level because of structural considerations.

M.3 Animal care and health

M.3.1 General

M.3.1.1 Unless there is good husbandry, veterinary or scientific justification for individual housing, animals should be maintained in compatible sociable groups. These groups should remain stable. Sheep and goats are herd animals which depend on social contact and will show severe stress reactions if separated from their flock. If individual housing is required, the animals should at least have visible contact with conspecifics.

M.3.1.2 Sheep display a leader-following behaviour. It is thus recommended to encourage this behaviour, for example, by moving sheep through gangways or scales instead of coercing the animals by means of force or fear, which usually results in chaos.

M.3.1.3 Sheep and goats also respond well to positive food reinforcement such as the provision of barley. Low stress handling can be achieved by competent, calm and confident personnel within an environment that is designed to assist such efforts.

M.3.2 Bedding material

M.3.2.1 With the exception of slatted floors, absorbent bedding material such as straw or wood shavings should be added to interior pens to provide a clean, comfortable and dry surface. A minimum average layer thickness of 10 cm of bedding material is recommended.

M.3.2.2 Bedding may be non-nutritive, but should be non-toxic, absorbent and comfortable. Resinous wood shavings, especially cedar, are not suitable for use as laboratory animal bedding. Pine shavings should be avoided for the same reason, although they are not as toxic as cedar.

M.3.2.3 Slatted floors or cages with grates or perforated bottoms require special caution. Care should be taken that the floors are specifically designed for the breed and weight class concerned, should provide secure footing, prevent injuries, and be comfortable.

M.3.3 Food and water (see table M.1)

M.3.3.1 Potable water should be supplied to animals in sufficient quantity and be presented in a manner that an animal can use. Water receptacles should be sited to avoid fouling, while still being accessible to young lambs and kids. Tap water might be sufficient for conventional housing facilities. Housing personnel should ensure that the height of the bunk- or trough-type feeder is suitable for the animals housed. Spaces between the vertical bars of feeders should not be between 75 mm and 180 mm to avoid trapping.

M.3.3.2 Where large numbers of breeding or stock animals are maintained in pens, it is important to ensure that there are sufficient feeding and watering stations to avoid undue competition (see table M.1).

Table M.1 — Minimum requirements for feeding and watering equipment for sheep and goats

1	2	3	4
Type of animal	Length of feed space per self-feeding animal cm	At least one trough number of animals	At least one nipple number of animals
Sheep			
Ewes	15	40	20
Ewes and lambs	15	30	15
Feeder lambs	10	50	25
Rams	20	10	5
Goats			
Does (nanny goats)	50	40	20
Young kids	30	50	25
Weaned kids	As per adult sex	As per adult sex	As per adult sex
Bucks (billy goats)	30	10	5

M.3.3.3 An individual animal's nutrient requirements are affected by many factors. Young animals generally need increased amounts of many nutrients. Reproduction places many demands on female animals, and nutrient requirements are very high in gestating and lactating animals. Environmental temperature and humidity can also affect food intake and nutrient needs.

M.3.3.4 All feed should be clean, free of contaminants or pests, palatable, fresh and sufficient for the animal's needs. The selected food should be a balanced diet that provides all required nutrients.

M.3.3.5 Feeding systems for goats should reflect goats' tendency to want to feed at head level or above or to climb into or onto feeders and other structures.

M.3.3.6 The technique of Body Condition Scoring (BCS) should be learned by all flock attendants to assess whether or not the diet of the animals in their care is maintaining the animals in good body condition.

M.3.4 Cleaning

M.3.4.1 Routine cleaning and maintenance, and a high standard of hygiene are essential for good husbandry. Suitable and institutionally approved cleaning agents and procedures should be applied.

M.3.4.2 The facilities should be designed to support manure removal, cleaning and disinfection.

M.3.4.3 Decisions on the frequency of cleaning should be based on the housing system, type of animal, stocking densities, and the ability of ventilation systems to maintain suitable air quality.

M.3.4.4 Fly, tick and other pest populations should be regularly monitored and appropriate control measures be applied when indicated.

M.3.5 Environmental enrichment

NOTE Little has been published on environmental enrichment strategies for sheep and goats, which might be because many studies on farm animals are carried out either in normal farm conditions or in similar conditions. Other studies remove animals only temporarily from normal farm conditions and return them after the intervention. However, particularly when farm animals are used in surgical experiments or as models for

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conditions other than normal farm conditions, environmental enrichment is not only important for the animal's well-being but also in order to obtain valid data.

The ability of sheep and goats to thrive on adverse rations and their adaptability reflect the considerable variability of environments in which they are found. This suggests that sheep and goats will respond positively to variations in their environment such as changes in feed, efforts to display grazing behaviour, or other enrichment items that provide a more stimulating environment.

The successful application of the use of positive reinforcement for sheep and goats has been described in Hutson GD 1985 and Hargraves and Hutson, 1990 (see bibliography).

M.3.6 Animal accommodation (see table 19)

M.3.6.1 Sheep and goat housing facilities should provide suitable access and restraining devices to allow animals to be inspected, caught or moved as necessary.

M.3.6.2 If sheep or goats are maintained over longer periods, hoof trimming should be part of the flock management programme. Such a programme should include shearing of sheep and goats where necessary.

M.3.6.3 Pens should be of sturdy construction to contain the animals securely and should be designed and maintained to prevent sheep or goats from becoming trapped or injuring themselves. This is of particular importance in the case of horned animals.

M.3.6.4 All materials used in pens to which sheep and goats have access, including paint and wood preservatives, should not contain any chemical substances known to be harmful to the flock.

M.3.6.5 Space allowances for sheep and goats vary greatly depending on animal size, fleece length, presence or absence of horns, gestation status, lactation status, climate conditions, etc. In general, pens should be large enough to allow all sheep and goats to lie comfortably on a dry and bedded area. During transport or when in other pens where sheep or goats are kept for short periods, enough space should be allowed for all animals to stand comfortably.

M.3.6.6 For specific purposes (for example, immediate post-operative care or metabolic studies) it might be justified to restrict the available space or other aspects of the primary enclosure (or both). Such studies should state these conditions clearly in the proposal to the AEC for it to be approved.

M.3.7 Breeding

M.3.7.1 Ewes and does should be allowed to give birth with minimum interference. Animal attendants should be familiar with normal birth and should be able to recognize problems. Assistance in birthing should, if necessary, be provided under veterinary supervision.

M.3.7.2 Newborn lambs and kids require adequate nutrition and a high level of hygiene. Mothers and their offspring should be disturbed as little as possible.

M.3.7.3 Aborting ewes and does, ewes and does at risk of aborting, and lambing ewes and kidding does might be infected with diseases potentially hazardous to pregnant females. It is recommended that females at risk should, in consultation with the veterinarian and physician, take the necessary precautions.

M.3.7.4 Detailed records should be kept of pedigrees as well as of fertility and rearing success.

M.3.8 Animal identification

M.3.8.1 General

M.3.8.1.1 The most important considerations in choosing a marking technique concern its effect on the behaviour, physiology and survival of the animal. Any technique that causes an adverse effect on the animal is not only inhumane, but is likely to distort the data being collected, resulting in meaningless and often misleading results.

M.3.8.1.2 In choosing an acceptable marking technique, the researcher should consider the nature and duration of restraint, the amount of tissue removed or damaged, whether or not pain, if inflicted, is momentary or prolonged, and whether the risk of infection and abcessation is minimal.

M.3.8.2 Permanent marking

M.3.8.2.1 Ear-notching provides an acceptable manner to number and thus uniquely identify sheep and goats. This should be carried out by an experienced operator, using properly maintained instruments and good hygienic technique.

M.3.8.2.2 Microchips are widely used to uniquely identify animals. New generation microchips even allow for the measuring of body temperature or the storage of animal data on the chip.

M.3.8.2.3 Ear-tags of a suitable size for small livestock are widely available and often used. More than two tags per ear is considered excessive. When reapplying tags, the operator should use the pre-existing hole(s) in the ear.

M.3.8.2.4 Tattoos on one or both ears may also be used. Tattooing should be carried out by an experienced operator, using properly maintained equipment and good hygienic practice.

NOTE Owing to their ease of identification and application, ear-tags have largely replaced tattoos.

M.3.8.3 Semi-permanent marking

A patch of fleece or patterns may be shaved, clipped or cut with pair of scissors. Such marks generally last from one week to four weeks (depending on the stage of the hair cycle) and can be used on any colour sheep or goat.

M.3.8.4 Temporary marking

Sheep and goats are often marked with marking sticks that leave a strip of colour on the coat. This is easily applied but only lasts for several days, and then it can be reapplied.

M.3.9 Handling

M.3.9.1 Like most animals in research facilities, sheep and goats respond best to gentle and firm handling. Sheep should be caught under the jaw or by the flank, or by the use of a crook. They should never be caught by grabbing their fleece. They should be held securely and, if the procedure allows it, kept with all four feet firmly on the ground. Sheep have sensitive skin and should therefore not be held by their fleece. If it is necessary, a sheep can be made to sit up on his or her hindquarters while the handler holds the forelegs and provides firm-and-gentle support to the head and back region with his or her legs and body.

M.3.9.2 People attending to goats should know how to correctly catch and restrain them. Goats should not be caught or moved by grabbing their fleece or hair. Catching them by the horns should be done with caution to avoid breaking the horns or damaging the skull. The use of a crook is

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acceptable. Goats should be restrained with one hand under the jaw and one hand over the head. The sitting technique used with sheep (see M.3.9.1) should not be used for goats since this can break their tails. Appropriate techniques might include the use of handling chutes or halters.

M.3.9.3 Sheep and goats should be lifted with proper support for the chest and abdomen and should not be lifted by the head, ears, horns, tail, legs or fleece.

M.3.10 Records

Regular monitoring of health and reproductive data, and keeping detailed records thereof, is essential to ensure that problems are identified at an early stage so that corrective action can be implemented to minimize any potentially adverse welfare effects on the animals. This form of monitoring and assessment is of particular importance in flocks, where large numbers of animals are maintained, or where there is a high animal turnover.

M.4 References

See bibliography.

Annex N

(informative)

Care and management of laboratory animals — Terrestrial reptiles

N.1 General

N.1.1 Reptiles are ectothermic (cold-blooded vertebrates incapable of metabolic thermoregulation and reliant on the external environment to control their body temperature) and are distinguishable by their dry scaly skin or shell (or both). This group includes snakes, tortoises, turtles, lizards, crocodiles and alligators. All breathe air by means of lungs at all stages of life. The homeostatic abilities of reptiles are far less well developed than mammals and under natural conditions they will select microenvironments in which they can gain or lose heat, as required, to maintain their optimal body temperature. The keratinized skin protects them from waterloss and from the absorption of noxious substances from their environment.

N.1.2 Virtually all major groups of reptiles contain some endangered species. National and international conservation regulations shall be considered and complied with when reptiles are held in captivity for study purposes. These regulations provide for the conservation, protection, survival and propagation of the animal species.

N.1.3 Reptiles are most commonly used for anatomical, physiological and behavioural studies. Most are captured in the wild and have a limited capacity to survive under captive conditions. Success in captivity depends largely on the ability of the keepers to create an acceptable simulated environment.

N.1.4 It is advisable to maintain the different species separately and keep the numbers held in primary enclosures to a minimum.

N.1.5 The primary goal in reptilian husbandry is to establish and maintain normal feeding and behavioural patterns and to reduce captivity stress. If successful maintenance and meaningful study data is to be achieved under captive conditions, reptiles should be provided with relevant temperatures, humidity, and light cycles that promote normal physiological and behavioural functioning of the species.

N.2 Behavioural thermoregulation

Little body heat is produced by the relatively low metabolic rates of reptiles. Owing to lack of insulation and sub dermal fat, heat is difficult to preserve in their bodies. External heat sources are vital for reptiles. Behaviour is adjusted to take advantage of heat or cooling sources. Basking involves distinct postures such as the flattening out of the body and orientation. In small lizards, the rates of heat gain and loss are rapid, and shuttling from shade to sun is frequent. The upper and lower thermoregulatory set points vary with each species, and in individuals of the same species. Understanding of thermoregulatory requirements is essential in the laboratory management of reptiles.

N.3 Behavioural interactions

N.3.1 Many species of reptiles are territorial and if individuals are kept together in laboratory cages they will form dominance hierarchies. Captivity does not allow the low status individuals to flee to alternative locations. Stress and physical injury will occur, leading to the reptile's exclusion from basking, feeding and retreat, and failure to thrive. This is particularly important in lizards.

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N.3.2 Increasing the spatial heterogeneity of the environment can ensure individuals encounter each other less frequently. Multiple basking, feeding and refuge sites should be provided.

N.4 Sensory systems

Most reptiles have colour vision, although snakes do not. Reptiles respond to visual stimuli and to direct movement. Direct olfaction is poorly developed but lizards and snakes have forked tongues that provide a "touch-smell" sense which enables them to obtain detailed information on the immediate environment and the presence of other individuals, including potential prey and predators. Venomous snakes use these chemical cues to follow bitten prey until their point of death.

N.5 Animal accommodation (see 7.6.13)

N.5.1 Housing for reptiles

N.5.1.1 Most snakes, lizards and the more terrestrial types of turtles can be held in terraria, which may be a modified aquarium or specially purpose-constructed housing.

N.5.1.2 Ideally, a species-specific designated holding room is required. However, the number of reptiles held in an animal facility is often insufficient to warrant species separation. Holding animals with different environmental requirements in a common room is manageable provided general conditions are established for the room as a whole. Individual terraria, cages and tanks may be set up as environmental chambers with independent control of temperature, humidity and light intensity levels suited to the individual species.

N.5.1.3 Small snakes do not do well in large cages. Large snakes do not do well in small cages.

N.5.1.4 Snakes are solitary by nature and do well when housed alone. Some may be housed in pairs or groups provided they are of similar size and are not cannibalistic. They should be separated during and immediately after feeding to prevent inadvertent ingestion of cage mates.

N.5.1.5 Snakes may be housed in glass, plexiglass aquaria or terraria, suitable plastics boxes, or specially constructed reptile cages. Cages should be impervious to water and should be able to be cleaned and disinfected.

N.5.1.6 Cages should have tight-fitting, secure lids with soft screen or holes to allow adequate air exchange. Most snakes can push off loose-fitting lids and can squeeze through very narrow openings. All doors, lids and screens should be fitted with latches, hooks or hasps.

N.5.1.7 The cage bottom can be lined with paper, indoor or outdoor carpet or shredded paper, or flat coarse wood shavings. Sawdust or other fine particle substrate should be avoided as it might be ingested with food. Mouth injuries, infections and bowel obstructions can result from the excessive ingestion of particulate substrates. This can be avoided by feeding dead food in a clean dish or on a solid clean surface. A change to different substrate might be required.

N.5.1.8 Aromatic wood shavings (for example, pine) can be toxic to snakes and should not be used.

N.5.1.9 A water bowl, large enough for the snake to crawl into, should be provided. Bowls should be heavy enough not to tip over and shall be able to be cleaned and disinfected. Snakes can spend a lot of time soaking, particularly at shedding time. Water containers should be changed and cleaned every 1 d to 2 d to avoid faecal contamination and bacterial build-up.

N.5.2 Aquatic holding systems for fresh water turtles

N.5.2.1 Water turtles are the most commonly held aquatic reptiles in the laboratory. Tanks and enclosures should provide sufficient space for normal movements and exercise patterns of the animals held.

N.5.2.2 Flow through fresh water systems used to supply regular fish tanks are suitable. Water levels required are less than for fish, but should be sufficient to allow turtles to completely submerge. A platform just clear of the water surface should be provided as a resting board on which to climb out onto. Wood may be used, but should not be painted or treated, and should be replaced at intervals. Water temperature should be held at 30 °C. A low wattage electric lamp can be provided above the basking platform to allow turtles to increase their body temperature as required.

N.5.3 Housing for venomous snakes

In addition to general housing requirements (see N.5.1) the following precautionary criteria should be met:

a) Ventilation ports

All openings, except the lid, should be covered with a double layer of screening to provide effective and added protection against bites. Screens should be a minimum of 1 cm apart to account for the length of the snake's fangs.

b) Viewing walls

Removable opaque covers should be fitted to the outside as many venomous snakes are irritable. Plexiglass walling is preferred as it ensures against shattering and escape. Irritable snakes might strike continually at the terrarium wall if disturbed and an opaque shield should be used to prevent external disturbances.

c) Access

Only the lid of the venomous snake terrarium should open. The terrarium should be deep enough to slow down any attempt by the snake to climb to the top. If floor level doors are used, it should be ensured that it is possible to see the snake before and while opening the door. The door should be hinged so as to provide a barrier between the inside of the terrarium and the handler.

d) Security precautions

A formal security and inspection system should be implemented to ensure that access is controlled and limited to authorized personnel only. Medical emergency phone numbers should be displayed in all areas.

e) Training and experience

Handlers working with venomous snakes should be appropriately trained and shall have suitable handling experience.

f) Antisera

The institution, animal facility and all medical staff shall be informed of the type(s) of snakes held or being introduced. Appropriate stocks of antisera for emergency use should be available on site, and personnel be trained in administering it.

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N.6 Skin and scales

N.6.1 The skin of terrestrial reptiles is composed of the dermis and epidermis (thickened and keratinized to form plate-like scales which can be overlapping). It is the outer part of the epidermis that is lost periodically in shedding (ecdysis). Replacement is from the deeper strata of the epidermis.

N.6.2 All reptiles periodically shed their skin, including the scales that cover and protect the eyes, usually in a single sheet. How quickly, completely and intact this process occurs is an indication of health status. More frequent shedding generally indicates a healthy eating and growing animal. Several days before shedding, the skin, and especially the eyes, becomes cloudy and opaque. Placing a snake in a bowl of warm water can assist the shedding process.

N.6.3 Lizards, turtles and crocodiles shed in many pieces.

N.7 Diet, UV radiation and mineral imbalances

Many reptile species in captivity are particularly susceptible to imbalances in mineral metabolism that results from a faulty diet, or insufficient exposure to UV radiation (sunshine). The most common reasons are:

- a) inadequate calcium in the diet;
- b) a high ratio of phosphorus to calcium;
- c) a dietary deficiency in vitamin D3; and
- d) inadequate exposure to UV radiation for the dermal synthesis of cholecalciferol (precursor of vitamin D3).

All of these situations can give rise to rickets, a soft shell, and a predisposition to disease.

N.8 Size range and lifespan

N.8.1 Adult reptiles range in size from lizards of less than 40 mm total length and weighing 1 gm to pythons of more than 9 m, and crocodiles of 6 m and more.

N.8.2 Smaller reptiles have a shorter lifespan. Lizards' lifespan varies between 1 year and 10 years with very large individuals reaching 30 years. Pythons and boas can live up to 30 years, and small tortoises up to 20 years, with larger individuals living up to a 100 years.

N.9 Reproduction and egg-laying

N.9.1 Fertilization in reptiles is internal and can be the outcome of an elaborate courtship behaviour. Many species of reptiles are sexually dimorphic as adults. Gentle probing of the cloaca can be used to determine the sex. If the probe cannot be inserted when directed caudally, then the animal is female. In males the probe will enter a sulcus that contains the hemi-penis.

N.9.2 Females of most reptile species lay eggs. In those which do not, the eggs are retained in the oviduct (ovoviviparity) until the young are ready for independent existence.

N.9.3 Egg-laying females will bury their eggs in soil or sand, rock crevices, or under bark.

N.9.4 Eggs are oval or round, and the shell covering can be hard (calcium salts) or relatively soft and leathery.

N.9.5 Temperature affects the rate of development. Humidity of the substrate is important. If substrate is too dry, the eggs dehydrate, and if too wet, the eggs absorb water and the embryo drowns or becomes infected with fungi or bacteria.

N.10 Species used in the laboratory

A wide range of reptiles, most of which are caught in the wild, may be kept in the laboratory. Conservation laws and regulations (including required permits) shall comply with the Provincial Nature Conservation Ordinance and Bio Diversity Act, 2004 (Act No. 10 of 2004).

N.11 Temperature and light

N.11.1 General

N.11.1.1 Most snakes need a warm ambient temperature and do well with a thermal gradient provided in the cage. A low wattage tungsten electric light bulb placed outside the cage and focussed on the basking surface will create the thermal gradient. Direct contact with any heat source (heating pads, lamps and electric bulbs) shall be avoided.

N.11.1.2 Cage temperature should be monitored daily to ensure the environment does not become too hot or too cold. Extremes in temperature can be fatal.

N.11.1.3 Optimal temperatures for reptiles are 25 °C to 30 °C, and for lizards up to 35 °C.

N.11.1.4 An independent heating source capable of operating when other lights are off, or in emergencies, should be provided. Generally 8 h to 10 h heating per day is sufficient since it is a regime that mimics the behaviour under natural sunshine.

N.11.1.5 If heating is switched on for excessively long periods, this will increase the animal's metabolic expenditure and food intake might be insufficient to compensate for this loss.

N.11.1.6 A regular light cycle should always be maintained, or be as determined by approved study requirements.

N.11.2 Ultraviolet radiation

N.11.2.1 Where exposure to natural sunlight is not possible, UV radiation should be supplied.

N.11.2.2 A wide range of fluorescent tubes that will supply this are commercially available, and the following considerations are important when using artificial UV light sources:

- a) UV emission from fluorescent tubes decreases with time (ageing).
- b) Exposure shall be direct since normal glass does not transmit UV radiation.
- c) The intensity of UV radiation attenuates rapidly. Tubes need to be close to the animals that are to benefit from them.
- d) Broad-spectrum tubes are of limited usefulness. Middle and long wavelength lights are optimal.

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- e) Tubes designed to promote plant growth (which have peak emission in the blue part of the spectrum) emit little useful UV radiation.
- f) Fluorescent and mercury vapour sunlamps used for tanning by humans are not to be used as they have the potential to cause retinal damage and skin burns.

N.12 Humidity and ventilation

N.12.1 Reptiles are better able to prevent waterloss from their bodies than amphibians. They can withstand lower humidity levels. However, low humidity can be hazardous for small lizards and those species adapted to humid, tropical conditions. These will require a constant humidity level of 60 %. Higher humidity can be achieved by evaporating water from a container placed near the heating source, provision of well-watered pot plants, and automated intermittent water mist sprayers in the terrarium. Ventilation ports may be covered with paper to reduce air exchange.

N.12.2 Control of air exchange in the terrarium as well as the holding room should be provided. Ventilation ports shall be screened to allow airflow and prevent escape.

N.12.3 Excessive humidity can lead to a predisposition to diseases of the respiratory system, skin, scales, and shells of tortoises.

N.13 Refugia

N.13.1 Most species of reptiles have periods of the day when they are not active and will seek some kind of refuge under rocks, in burrows, in crevices, under bark, in trees, bushes or dense vegetation, or in water.

N.13.2 Failure to provide refugia for captive animals results in high stress levels. Refuge that mimics the natural environment of the specific species should always be provided. The internal arrangement of a cage once the animal has adapted to it should not be altered. Some lizards prefer the refuge to be close to the basking area.

N.13.3 Snakes are shy by nature and should be able to periodically hide from view. Terracotta flowerpots, rock caves, sections of tree bark, and commercial hide boxes provide good retreats. Snakes prefer opaque to transparent walls.

N.13.4 A raised flat surface, with a heat source for basking on, should be provided for all reptiles.

N.14 Servicing and viewing

N.14.1 Terrarium doors and lids should, with the exception of those housing venomous snakes, be constructed so that the entire top, or an end or side, can open up for cleaning.

N.14.2 An opaque top and three opaque sides is generally preferred, otherwise reptiles should be provided with a covered refuge area where they can shield from light and disturbance. In the case of frightened or highly irritable species, the viewing panel may be covered with a removable screen.

N.15 Sanitation

N.15.1 Reptiles have relatively low metabolic rates and produce fairly small quantities of faecal material. Nitrogenous excretion is mostly in the form of insoluble uric acid.

N.15.2 Tortoises and herbivorous lizards have the bulkiest faeces. Some faeces contain pheromones and are used for communication. Too frequent cleaning can adversely affect this. Leaving a small amount of faeces after cleaning might reduce the inclination to escape.

N.15.3 Cages should be impervious to water and cleaning agents shall allow proper disinfection. For most species, cages should be cleaned every one week to two weeks. A dilute bleach solution (1:30) is effective, but care should be taken to thoroughly remove any disinfectant residue by rinsing. Phenolic and cresolic compounds are very toxic to reptiles.

N.16 Water, food and feeding

N.16.1 General

N.16.1.1 Most snakes, lizards and terrestrial turtles need standing water. Most snakes will submerge themselves in water and containers should be large enough to allow this habit (see N.5.1.9).

N.16.1.2 For very small snakes and lizards, the shallow water dish should contain a water-soaked sponge or absorbent cotton to reduce the hazard of the animal becoming trapped in the container.

N.16.1.3 Snakes are totally carnivorous and swallow their prey whole. Many smaller species of snakes will eat mice. Larger species prefer rats. The majority of reptiles are predators with extremely narrow and specific diet specialities. There is a strong behavioural bias towards visual recognition of their prey, which can be a reason for some showing aversion to feeding on dead animals.

N.16.1.4 Most snakes will accept pre-killed prey, therefore it should not be necessary to feed live rodents. Frozen food items should be thawed and warmed to room temperature or slightly higher before feeding. Cold food items will putrefy rather than digest in the snake's stomach. Adult snakes will eat once weekly on average, but young snakes often require more frequent feeding (twice or three times weekly).

N.16.1.5 Some of the more highly irritable reptiles find captive conditions very stressful and will not feed at all, or will only do so under conditions of total isolation and privacy. Feed intake cannot be monitored efficiently under such conditions. Feeding may only take place in the dark, and the food should be warmed, or should be from a freshly-killed carcass.

N.16.1.6 Turtles lack teeth but they have a horny beak that is used for grasping and tearing food. Diet depends on the species.

N.16.1.7 Anorexia due to stress and inability to adapt to the less suitable environment in captivity will result in progressive weakness, emaciation and possible death. Force-feeding should be initiated well before the animal becomes emaciated and environmental conditions should be modified to encourage natural feeding patterns.

N.16.1.8 Many reptile illnesses are caused by improper diet or food preparation.

N.16.2 Carnivorous turtles

N.16.2.1 Snapping and pond turtles generally eat aquatic invertebrates, fish and frogs. In captivity, all will take dead food, whole fish, fillet, liver and meat.

N.16.2.2 In captivity, turtles, especially juveniles, are prone to calcium deficiencies caused by imbalances in the calcium to phosphate ratios and lack of vitamin D, which can result in metabolic

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osteopathies (for example, nutritional osteodystrophy), shell softening and general lethargy. These deficiencies are difficult to correct once they have occurred. Meat without bone provides an inadequate source of calcium.

N.16.3 Omnivorous and herbivorous turtles

Terrestrial turtles (tortoises) are omnivorous and will feed on a mixture of soft fruits and leafy green vegetables, as well as mealworms, insect larvae and adults. The calcium to phosphorus ratio in the diet should be examined.

N.16.4 Carnivorous and insectivorous lizards

Most lizards are insectivorous and have adapted to specific prey types. They can be fed on trapped insects, fruit, house and stable flies, insect larvae or nymph stages, and occasionally on earthworms.

N.16.5 Herbivorous lizards

The herbivorous species of lizard are generally those of the iguana species and will take soft pulpy fruits and leafy green vegetables.

N.17 Identification

Many individual reptiles can be recognized by a combination of their size, colour, and patterns. In snakes, clipping one of the ventral scales is effective but proper handling is required. Photographic or video records are often used. Use of microchips is recommended where practical.

N.18 Reproduction (Temperature and substrates)

Snake eggs may be removed from the cage and incubated in a warm, humid, and soft substrate environment. Eggs should not be rotated during incubation.

N.19 Diseases and parasites

N.19.1 General

N.19.1.1 Many snakes and reptiles are easy to keep. However, animals caught in the wild can carry viral, bacterial and parasitic diseases.

N.19.1.2 Improper feeding, sanitation, temperature, and other stress-producing factors will predispose to the development of illness and disease. Illness in snakes is characterised by open-mouth breathing, vomiting, diarrhoea, loss of appetite, and weightloss.

N.19.1.3 Aquatic reptiles are prone to superficial bacterial infections and, to a lesser extent, fungal infections. Signs of illness in turtles are:

- a) open-mouth breathing;
- b) swollen eyes;
- c) nasal discharge;
- d) blowing bubbles from the mouth or nose;

- e) soft shell;
- f) lethargy;
- g) loss of appetite; and
- h) diarrhoea.

Water soluble antibiotics, such as tetracyclines, are effective for bacterial infections. Superficial fungal infections are controlled by using daily bathing with potassium permanganate solution of 1:100,000 for 4 d to 5 d, nystatin, fungicidin and povidone iodine.

N.19.1.4 Systemic bacterial infections can arise because of dirty tanks and poor hygiene. Bacteria, such as *Pseudomonas* and *Aeromonas*, that thrive in the water are a major threat. Signs of disease are variable and often non-specific, with lethargy being the most noticeable. Often animals are found dead with no prior signs or warning of ill health.

N.19.1.5 The following infections can occur:

a) ***Salmonella***

Salmonella is carried by many reptilians and is a common and potentially serious zoonotic disease for humans. Captive reptilians should be checked for the presence of this organism.

b) ***Protozoan parasites***

Cryptosporidium spp. is increasingly found under laboratory conditions and treatment can be difficult. Strict hygiene is essential.

c) ***Parasitic worms***

All of the major groups of parasitic worms are found in reptiles. Those with intermediate host requirements (for example, tapeworms and flukes) are much less common. Nematodes have a direct lifecycle and can increase rapidly under ideal conditions. They may be readily treated with thiabendazole, fenbendazole, bunamidine hydrochloride, niclosamide, and praziquantel for tapeworms.

N.19.1.6 Newly introduced animals from the wild or other sources should be tested and quarantined for a minimum of 14 d.

N.19.2 Mite and tick infestations

N.19.2.1 Mite and tick infestations often arise from eggs surviving in the bedding substrate and are frequently encountered on snakes and lizards. They will multiply rapidly in warm conditions. Heavily infested animals show dusty white areas on the skin surface around the folds on the neck and legs.

N.19.2.2 Mites can be treated with a topical insecticidal dust, or application or injection of ivermectin. Substrates shall be replaced after the sanitation of the terraria, which should be left uninhabited for one week. Strips that contain dichlorvos can be placed out of animal reach in the terraria.

N.19.2.3 Ticks are often found attached to soft skin areas between scales and may be removed manually.

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N.19.3 Mouth rot (Necrotic stomatitis)

N.19.3.1 Mouth rot is a bacterial infection of the oral mucosa in snakes that often arises from injury during feeding, or accidental ingestion of substrate. Initially the inner surfaces of the lips and gums are affected by the bacterial infection and then followed by necrotic ulceration of the affected areas. (Inflammation is sometimes followed by petechial haemorrhages, thick caseous exudate and ulcerations under the exudate.) Severely affected animals usually die. Necrotic tissue needs to be removed under anaesthesia, cultures made from the affected site(s), and the appropriate antibiotic used.

N.19.3.2 Changing the substrate is recommended.

N.20 Handling and restraint

N.20.1 Appropriate handling equipment and protective clothing, gloves, goggles, plastics tubes, hooks and tongs should be available on site.

N.20.2 To inject snakes, a plastic tube of compatible diameter with predrilled holes for a hypodermic needle insertion is recommended. The snake should not be able to turn around in the tube.

N.20.3 Reptiles should be approached and handled calmly, gently, confidently and quickly. Hesitant or jerky movements can provoke a bite. When restraint of the head is necessary, it is important to comfortably support the reptile's whole body and legs. This prevents the tail from thrashing and becoming injured.

N.20.4 If a hook is used to transfer a snake to another container, this should be slid under the snake midway down the body. The snake is then lifted quickly \pm 1 m above the ground and transferred.

N.20.5 Highly venomous snakes should be handled as little as possible and only by experienced or specially trained personnel. Frightened snakes might strike and bite and let go immediately. Hungry snakes are more prone to bite and hold on.

N.20.6 Small lizards should not be enclosed in the hand for more than a few seconds as this can cause injury or asphyxiation. Tail damage in lizards is common, and handling should be reduced to essential procedures only.

N.21 Euthanasia

N.21.1 Freezing and simple decapitation are unacceptable methods. Decapitation poses the possibility for a reptile to retain consciousness for some period after the process.

N.21.2 Acceptable methods are exposure to halothane, methoxyflurane and injection of sodium pentobarbitone. Death should always be confirmed.

N.22 Health precautions for handlers

Snakes and other reptiles can carry *salmonella* which is transmissible to humans. Good hygiene and use of protective clothing will protect against this disease. Hands should always be washed thoroughly after handling reptiles.

N.23 References

See bibliography.

Annex O

(informative)

Pain management and humane endpoints

O.1 General

O.1.1 When conducting experiments that could involve a great deal of animal distress, one should consider the option of implementing humane endpoints.

O.1.2 The implementation of a humane endpoint should be a predicted well founded assessment of the welfare of the animal that is based on predetermined indicators such as tumour size or weightloss. Certain clinical signs can be evident of an irreversible process that will most probably lead to severely reduced welfare and, as such, these signs are an indication for a humane endpoint.

O.1.3 When preparing a project application for all but the most minor manipulations, the researcher or teacher should develop humane study endpoints. For animal welfare reasons, these can be used to judge when an animal requires to be put to death by recognized euthanasia methods. Death as an endpoint is generally ethically unacceptable and should be fully justified.

O.2 Why humane endpoints

The following are instances that render humane endpoints as acceptable:

a) **Moral consideration**

When the laboratory animal experiences more pain, suffering or chronic distress than was originally anticipated or is justified.

b) **Scientific consideration**

1) When the scientific objective of the experiment has been accomplished and keeping the animal does not contribute to the results of the investigation or even interferes with the results, or it is clear that the objective of the experiment cannot be achieved.

2) When keeping the animals can lead to loss of data (for instance, if it dies in the cage and the animal is subsequently cannibalized by cage mates, or the animal or organs or tissue are autolysed).

O.3 Types of criteria for humane endpoints

Humane endpoints can be based on different types of parameters (see table O.1). Globally speaking, these parameters can be grouped into the following categories:

- a) clinical behaviour (tumour formation);
- b) pathophysiological indicators (drop in body temperature);
- c) serious behaviour indicators (stereotypic behaviour) ;
- d) biomedical indicators (ketonuria); and
- e) hormonal indicators.

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Table O.1 — Some indicators used to justify humane endpoints

1	2	3
Clinical behaviour	Pathophysiological indicators	Biomedical or hormonal indicators
Activity Aggression Posture Response to handling Vocalization	Respiration rate Complete blood count Weightloss Heart rate Dehydration Anuria	Acute phase proteins Catecholamines Corticosteroids Glucagon Insulin Prolactin

O.4 The use of humane endpoints in research projects

O.4.1 In the design of a research protocol, thought should be given to the implementation of humane endpoints. Information pertaining to this issue should be laid down in the protocol and should include the following points:

- a) the clinical course, including any critical times and signs, and any anticipated discomfort or pain;
- b) observation frequency and the recording of the findings;
- c) the humane endpoint and the parameters underlying the establishment of the humane endpoint;
- d) the responsibilities of the person(s) involved in the observation, treatment or euthanasia;
- e) the type of alleviative treatment or euthanasia; and
- f) the postmortem procedure.

O.4.2 Should there be any doubt as to the (clinical) progression of the illness or about the parameters for determining the humane endpoint, then conducting a pilot study with a limited number of animals is recommended.

O.5 Humane endpoints and actions to be taken

O.5.1 Humane endpoints (see 3.10) are study-specific criteria that indicate or predict pain, distress or death and are used as signals to end a study early to avoid or terminate pain or distress (or both).

O.5.2 Once an animal reaches the specified humane endpoint, the veterinarian and the principal researcher should be informed, without delay, to make the decision to put the animal to death by recognized euthanasia methods, as well as any other decisions that could become necessary.

O.5.3 Specific actions should be taken, using recognized euthanasia methods, when

- a) an animal shows signs of a coma within 24 h to 48 h of the start of the experiment,
- b) an animal weighs less than its initial weight after 7 d or loses more than 20% of its initial weight at any time, or
- c) an animal shows tiptoe or slow ponderous gait.

If more than one clinical sign occurs, then the veterinarian and principal researcher should be informed.

O.6 Responsibility

O.6.1 Before the start of an animal experiment, all staff directly involved in the experiment need to be accurately informed about the critical period in the experiment by the principal researcher. All personnel should be knowledgeable about the following aspects:

- a) normal behaviour and physiology of the animal;
- b) anticipated deviations from the normal in the proposed procedure;
- c) awareness of their role and responsibility;
- d) the consultant in the event of unanticipated clinical effects;
- e) the moment at which a humane endpoint will be implemented;
- f) facilities and options for postmortem examination to establish the cause of death; and
- g) a scoring system to facilitate decision making (when to report deviations from the normal and to whom).

O.6.2 In a case of uncertainty, expert advice should be obtained, normally from a laboratory animal scientist, veterinarian, or a pathologist.

O.6.3 It is important that all responsible personnel be reachable at all times for consultation should questions arise concerning the implementation of a humane endpoint for an animal.

O.7 Pilot study

Pilot studies should be carried out before the main experiment to allow for the definition of various elements and parameters in the study. Pilot studies for setting humane endpoints in an experiment are needed when

- a) the effects of the treatment are unknown, so that morbidity, time course of effects, and specific clinical signs still have to be more narrowly defined,
- b) the identification of humane endpoints on the basis of specific parameters (for example, telemetrically obtained data) is possible, and
- c) the pathological changes observed can be used later to set humane endpoints.

O.8 Recognition

Adverse effects experienced by animals during experimentation include more than pain since they include conscious emotions such as fear, discomfort, distress (stress with which an animal fails to thrive or cope) (see Morton, 1998b) and mental distress (for example, frustration and boredom). However, before any of these states can be alleviated or assessed, or experiments refined in any way so as to cause less pain and suffering, there should be recognition of when the animal's wellbeing is being affected, both positively and negatively. Recognition can be considered as a fourth R, following on after the three As (Avoidance, Assessment and Alleviation) of animal suffering in research.

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It is important to eliminate any animal suffering in order to achieve scientific research of a high quality, specifically in relation to the scientific research questions being asked, as well as to practise humane scientific research economically (see Claasen, 1994 and Balls *et al*, 1995). Problems can be approached by using clinical signs as a way of determining the degree to which an animal's physiology and mental state have deviated from the normal. This is applicable not only to mammals, but to vertebrates and even non-vertebrates, provided there is suitable knowledge regarding their normal ethology and physiology.

O.9 Development and validation of humane endpoints

O.9.1 Planning considerations

O.9.1.1 Careful planning and implementation of humane endpoints requires a certain measure of expertise. Before the start of the experiment, its course should be anticipated and a decision about when to implement a humane endpoint should be reached.

O.9.1.2 The exact time of the endpoint is dependent on the objective of the experiment, but it should be chosen before the outset of any pain, distress or as soon as possible thereafter.

O.9.1.3 The endpoint should preferably be chosen on the basis of objective criteria. The moral and scientific considerations relating to humane endpoints (see O.2.(a) and O.2.(b)) should be kept in mind.

O.9.1.4 Before arriving at a suitable endpoint, the following preliminary stages should be undertaken:

- a) setting of priorities;
- b) a test analysis;
- c) identification and evaluation of potential endpoints;
- d) validation of selected endpoints; and
- e) approval by the AEC.

O.9.2 Criteria for endpoints

Ideally the endpoint should:

- a) be easy to monitor;
- b) be reproducible;
- c) not be labour intensive;
- d) in some cases, show valid prediction of the lethal progression of the illness;
- e) be relevant (equivalent) and reliable (with little variation);
- f) take intermediary steps towards an ultimate *in vitro* alternative; and
- g) show maximal reduction of pain and discomfort.

O.9.3 Validation

In practical terms, the following three steps should be taken to arrive at suitable humane endpoints:

- a) objective definition and recording of signs of pain and distress in the experiment;
- b) selection based on the significance of the signs in O.9.3 (a); and
- c) assurance of the scientific validation (i.e. it satisfies to a large degree the criteria in O.9.2).

O.10 Score sheet system

O.10.1 General

O.10.1.1 Score sheets should be drawn up specifically for each scientific procedure, and for each species undergoing that procedure. They can rarely be generalised.

NOTE The score sheet lists the clinical signs that are observable and measurable and are developed through the experience of a team of observers.

O.10.1.2 The team of observers are usually:

- a) animal caretakers since they are most likely to know when an animal is "not right" which will often indicate a change in behaviour, posture, appearance or even the feel or smell of an animal;
- b) veterinarians since they are skilled in identifying objective clinical signs and should have knowledge of the biology of the species, including the range of its relevant behavioural and physiological responses; and
- c) scientists since they should be conversant with the perturbations that might be expected during an experiment due to the scientific paradigm.

O.10.1.3 All the factors in O.10.1.2 will be important guides in the assessment of the effects of a scientific procedure on an animal. By detailing the cardinal signs of any particular protocol and regularly observing animals at critical periods during the experiment, an objective assessment of animal wellbeing can be made throughout the experimental period.

O.10.2 Method used to draw up and interpret a score sheet system

O.10.2.1 A list of signs is developed by closely observing the first few animals undergoing a novel scientific procedure. The list is then modified with experience until a set of cardinal signs that most animals will show during that experiment, which are relevant to the assessment of suffering, is obtained.

O.10.2.2 These clinical signs (see O.10.2.1) are set out against time in the score sheet.

O.10.2.3 Crucially, any clinical sign has to be reduced to a level which reduces the scope for observer interpretation and can only be recorded as being present or absent. This is indicated by a plus (+) or a minus (-) sign (sometimes a \pm sign if the observer is unsure). The convention is that negative signs indicate normality, i.e. within the normal range, and positive signs indicate that the animal is outside the normal range. In this way, it is possible to scan a score sheet to gain an overall impression of animal's wellbeing in that the more plusses, the more an animal has deviated from normality with the inference that it is suffering more than before.

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O.10.2.4 Animals should be scored during critical periods when they predictably could give rise to concern (for example, in the immediate post-operative period or in a study on infection after the incubation period).

O.10.3 Completion of the score sheet system

O.10.3.1 Practically, it is important to develop a disciplined strategy for the recognition of adverse effects in animals.

O.10.3.2 At the beginning of an assessment, the animal should be viewed from a distance, and its natural undisturbed behaviour and appearance noted.

O.10.3.3 Next, as the observer approaches the pen or removes the cage lid, the animal will inevitably start to interact with the observer and its response can be used to determine whether it is normal or abnormal.

O.10.3.4 Finally, a detailed clinical examination can be carried out by handling and restraining the animal and observing its appearance carefully as well as making any relevant clinical measurement (for example, bodyweight and temperature).

O.10.3.5 At the end of the score sheet there should be guidance notes for animal caretakers, veterinarians or laboratory animal technologists about:

- a) what should be provided in terms of husbandry and care for animals undergoing that scientific procedure;
- b) how to record qualitative clinical signs (such as diarrhoea and respiration); and
- c) criteria by which to implement humane endpoints.

O.10.3.6 If an animal has to be killed, there should be instructions about any other actions that should be taken, such as tissue to be retrieved or placed in 10 % formaldehyde in saline (see O.10.4.3). This helps ensure that the maximum information is obtained from any animal in a study.

NOTE Although these score sheets take time to fill in, it is not difficult for an experienced person to see if any animal is unwell so the NAD (Nothing Abnormal Detected) box is simply checked.

O.10.4 Score sheet

O.10.4.1 Special husbandry requirements

Special husbandry requirements should be stipulated before the start of the observation, for example:

- a) animals shall be fed an irradiated diet and adapted to it 2 d to 3 d before diabetes induction;
- b) animal cages shall be cleaned out twice daily;
- c) two bottles of UV water shall be provided for each cage and filled twice daily; and
- d) animals shall be deprived of water overnight.

NOTE Deprivation of water should not be sufficient to cause death by dehydration.

O.10.4.2 Interpretation

A sample score sheet developed to record clinical signs for rats with streptozotocin-induced diabetes is given in table O.2. It can be seen from this sample score sheet that there are more plus signs on the right hand side (see O.10.2.3). Several other points should be noted as follows:

- a) When the animal started to show clinical signs, it was scored more frequently.
- b) During day 0 (the day of the injection of streptozotocin to induce diabetes), the animal lost body weight due to the restricted food intake the previous night.
- c) Over the next 2 d, the animal lost body weight although it was normal in all other respects.
- d) By day four, the coat became starey (ruffled), the body temperature had dropped significantly, and the breathing had become more rapid and laboured.
- e) Furthermore, there was a significant body weightloss (22 %) which is a strong indication that the animal had not eaten or drunk much or that it was not maintaining its fluid balance, and tiptoe walking indicated some degree of abdominal pain. The rapid weightloss and dehydration, laboured breathing, abnormal posture, among other signs, all confirmed that the animal was becoming severely physiologically compromised and was not going to yield valid results in relation to the scientific objective.
- f) Even more significantly, the animal's temperature dropped, which is a very poor sign.
- g) The animal was given fluids, placed in a warm environment, and observed 3 h later.
- h) The animal was not responding adequately and, from experience from following such animals through to death in earlier studies, it would have died that night if not sooner.
- i) It was consequently decided that the animal be put to death by recognized euthanasia methods on humane and scientific grounds before the end of the experiment. Where an ethical balance is struck between the anticipated benefits of a research project and the degree of animal distress (as indicated by the humane endpoints), the severity limit had been exceeded. Even if the animal might not have died, the level of pain and distress was agreed to be a sufficient reason to put the animal to death by recognized euthanasia methods on humane grounds alone.

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Table O.2 — Sample score sheet to record clinical signs for rats with streptozotocin-induced diabetes

Date of study: 2007-08-09		Animal No.: Rat 3				
Weight (g):						
Date	9 Aug	10 Aug	11 Aug	12 Aug	13 Aug	14 Aug
Day	0	1	2	3	4	4
Time	8:40	9:00	8:50	8:55	8:05	11:00
FROM A DISTANCE						
Fed	Y	Y	Y	Y	Y	Y
Inactive		-	-	-	+	+
Isolated		-	-	-	-	-
Walking tiptoe		-	-	-	+	+
Hunched posture		-	-	-	+	+
Pinched face		-	-	-	+	+
Ruffled coat		-	-	-	+	±
Type of breathing ^a		N	N	N	120 L	70 L
ON HANDLING						
Not inquisitive and alert		-	-	-	+	+
Not eating		-	-	-	±	+
Not drinking		-	-	-	?	+
Vocalization on gentle palpation		-	-	-	-	-
Volume water drunk by average of rats in cage (ml)		50	113	133	140 av	0
Body weight (g)	204	209	203	192	170	168
Percentage change from pre-starved weight	7	5	7	12	22	
Body temperature (°C)		37,5	37,4	37,6	32,4	34,7
Pale or sunken eyes		-	-	-	+	+
Dehydration		-	-	-	+	+
Distended abdomen/swollen		-	-	-	±	±
Diarrhoea ^b 0 to 3 (+m or +b)		-	-	-	-	-
Cage wet		-	±	+	-	-
Condition grading 4 to 1 ^c		4	4	3	2+	2
Saline given s/c – volume/site?		-	-	-	2 mlx 2	-
Blood sugar level		nd	nd	nd	nd	nd
Nothing Abnormal Detected (NAD)		-	-	-	-	-
OTHER						
SIGNATURE:						
Scoring details:						
^a Breathing: R = rapid; S = shallow; L = laboured; N = normal. ^b 0 = normal; 1 = loose faeces on the floor; 2 = pools of faeces on the floor; 3 = running out on handling. (+m = mucus and +b = blood). ^c Condition: 4 = normal; 1 = emaciated. nd = not determined.						

O.10.4.3 Scientific measures

Instructions for scientific measures should be followed, for example, a kidney should be placed into a mixture of 10 % formaldehyde in saline.

O.10.5 Some advantages of the score sheet system

The score sheet system used to record clinical signs for the recognition and assessment of adverse effect on animals during scientific procedures has been shown to have the following advantages:

- a) closer observation of animals can now be carried out by all staff at critical times in the experiment as the score sheets indicate the times when animals find their circumstances most aversive;
- b) subjective assessments of suffering by staff and researchers are avoided, thereby promoting more fruitful dialogue, as evidence based on opinion becomes possible supported by the clinical proof;
- c) consistency of scoring is increased as the guidance is clear and the scoring options are limited;
- d) single signs or combination of signs can be used to indicate overall severity of the procedure, as well as alleviative therapies or scientific procedures as set points in an experiment (for example, blood sampling); and
- e) the score sheet system:
 - helps determine the effectiveness of any therapy intended to relieve adverse effects;
 - can be used to determine which experimental models cause the least pain and distress (for example, by comparing alternative animal models), thus helping to refine scientific procedures;
 - can be used to analyze retrospectively the adverse effects of any scientific procedure and its severity level;
 - has been found to add to the scientific study as a more careful observation of animals is carried out;
 - provides a visual aid, opens up discussion between interested parties, and helps focus attention on an animal's condition throughout the procedures. Any analysis of the score sheet can reveal patterns of recovery or deterioration and so gives a better picture of the effect of a procedure on animals from start to finish. The sheet encourages all involved to observe the behaviour of animals and to recognize normal and abnormal behaviour, thus helping in determining animal responses to various procedures which will help to devise ways of refining experimental techniques by highlighting the type and timing of any adverse effects. The score sheets are constantly being developed and updated with further experience. Staff also start to perceive patterns of adverse effects that, when taken as a whole, indicate early death or early deterioration sufficient to warrant the animal being killed on scientific grounds alone. Such information leads to better animal care as well as provides useful scientific information such as the recognition of neurological deficit, times of epilepsy or weightloss, as well as unexpected findings. Furthermore, by picking up signs of poor animal wellbeing early, humane endpoints can be implemented sooner rather than later and so avoids animals being inadvertently lost from an experiment through unexpected death (see Redgate *et al*, 1991; Olfert, 1995; Soothill *et al*, 1993; Townsend & Morton, 1994; Mellor & Morton, 1997; Cussler *et al*, 1998; U1KCCCR, 1998); and

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- has proved to be especially useful with new procedures or when users are not always sure of what effects a procedure will have. Literature rarely records adverse effects on animals or how to avoid or measure them. Only researchers have a moral obligation to do so (see Morton, 1998b).

O.11 Score sheets

O.11.1 Score sheets for specific research

Further examples of score sheets for specific research are as follows:

- a) cancer research (see table O.3);
- b) toxicity studies (see table O.4);
- c) vaccine quality control (see table O.5); and
- d) infectious disease research (see table O.6).

O.11.2 Cancer research

O.11.2.1 Special husbandry requirements

Special husbandry requirements should be stipulated before the start of the observation, for example:

- a) after the tumour becomes visible, the frequency of observation and sizing of the tumour shall be increased; and
- b) particular attention shall be paid to the growth rate of the tumour.

Table O.3 — Score sheet for cancer research

Date of study		Animal No.:					
Weight (g)							
UNDISTURBED OBSERVATION							
Date							
Day							
Time							
Inactive							
Mobility							
Hunched posture							
Grooming							
Alertness							
Presence of a growth							
Ruffled coat							
ON HANDLING							
Not inquisitive and alert							
Not eating							
Not drinking							
Vocalization on gentle palpation							
Body weight (g)							
Percentage of baseline weight							
Dehydration							
Type of breathing ^a							
Condition grading 4 to 1 ^b							
SPECIFIC CLINICAL SIGNS							
Size of tumour							
Necrosis of tumour							
Bleeding of tumour							
Ulceration							
Nothing Abnormal Detected (NAD)							
OTHER							
SIGNATURE							
Scoring details:							
^a Breathing: R = rapid; S = shallow; L = laboured; N = normal.							
^b Condition: 4 = normal; 1 = emaciated.							
nd = not determined.							

O.11.2.2 Humane endpoints or actions

O.11.2.2.1 If more than one (negative) clinical sign occurs then the veterinarian and the principal scientist should be informed.

O.11.2.2.2 Humane endpoints or actions should be taken, using recognized euthanasia methods, when

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- a) an animal weighs less than its initial weight after 7 d or loses more than 10 % of its initial body weight at any time,
- b) before a tumour reaches a predetermined size,

NOTE In general, the tumour should not exceed 10 % of the body weight. For a mouse, the appropriate maximal diameter is 2 cm.

- c) an animal is in a poor condition (condition grading of 1 = emaciated), and
- d) there is necrosis or bleeding of the tumour.

O.11.2.3 Scientific measures

Instructions for scientific measures should be followed.

O.11.3 Toxicity studies

O.11.3.1 Special husbandry requirements

Special husbandry requirements should be stipulated before the start of the observation, for example:

- a) the animals shall be observed several times a day;
- b) husbandry and nutritional needs shall be met and be compatible with scientific requirements; and
- c) the responses of animals to housing and husbandry regimes during their active period shall be monitored.

Table O.4 — Score sheet for toxicity studies

Date of study		Animal No.:					
Weight (g)							
Date							
Day							
Time							
UNDISTURBED OBSERVATION							
Inactive							
Mobility							
Hunched posture							
Grooming							
Alertness							
Presence of a discharge							
Ruffled coat							
ON HANDLING							
Not inquisitive and alert							
Not eating							
Not drinking							
Vocalization on gentle palpation							
Body weight (g)							
Percentage of baseline weight							
Dehydration							
Type of breathing ^a							
Condition grading 4 to 1 ^b							
SPECIFIC CLINICAL SIGNS							
Behavioural changes							
Tremors							
Circling							
Convulsions							
Comatosed							
Nothing Abnormal Detected (NAD)							
OTHER							
SIGNATURE							
Scoring details:							
^a Breathing: R = rapid; S = shallow; L = laboured; N = normal.							
^b Condition: 4 = normal; 1 = emaciated.							
nd = not determined.							

O.11.3.2 Humane endpoints or actions

O.11.3.2.1 If more than one (negative) clinical sign occurs then the veterinarian and the principal scientist should be informed.

O.11.3.2.2 Humane endpoints or actions should be taken, using recognized euthanasia methods, when

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- a) there is a case of convulsions, or
- b) there is severe weightloss (>20 %) and dehydration.

O.11.3.2.3 The time of euthanasia shall be decided by the responsible laboratory animal or veterinary technologist.

O.11.3.3 Scientific measures

Instructions for scientific measures should be followed.

O.11.4 Vaccine quality control

O.11.4.1 Special husbandry requirements

Special husbandry requirements should be stipulated before the start of the observation, for example, animals shall be assessed twice per day until the end of the experiment.

Table O.5 — Score sheet for vaccine quality control

Date of study								Animal No.:
Weight (g)								
Date								
Day								
Time								
UNDISTURBED OBSERVATION								
Inactive								
Mobility								
Hunched posture								
Grooming								
Alertness								
Presence of a discharge								
Ruffled coat								
ON HANDLING								
Not inquisitive and alert								
Not eating								
Not drinking								
Vocalization on gentle palpation								
Body weight (g)								
Percentage of baseline weight								
Dehydration								
Type of breathing ^a								
Condition grading 4 to 1 ^b								
SPECIFIC CLINICAL SIGNS								
Central nervous signs and specifications								
Body temperature (°C)								
Nothing Abnormal Detected (NAD)								
Number of animals put to death by recognized euthanasia methods or that died								
OTHER								
SIGNATURE								
Scoring details:								
^a Breathing: R = rapid; S = shallow; L = laboured; N = normal.								
^b Condition: 4 = normal; 1 = emaciated.								
nd = not determined.								

O.11.4.2 Humane endpoints or actions

O.11.4.2.1 If more than one (negative) clinical sign occurs then the veterinarian and the principal scientist should be informed.

O.11.4.2.2 Humane endpoints or actions should be taken, using recognized euthanasia methods, when

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- a) there are central nervous signs such as ataxia or convulsions,
- b) a low body temperature (<34,5 °C) is observed, or

NOTE Validation studies have shown that a drop in body weight is not always predictive of a lethal outcome.

- c) a decision by the responsible laboratory animal veterinary technologist to terminate is taken.

O.11.4.2.3 For each animal group, the number of animals that die per day should be recorded.

O.11.4.3 Scientific measures

Instructions for scientific measures should be followed.

O.11.5 Infectious disease research

O.11.5.1 Special husbandry requirements

Special husbandry requirements should be stipulated before the start of the observation, for example:

- a) cage sanitation schedules should be altered to accommodate special research needs;
- b) cages and waste pans or trays should be sanitized weekly or more often, if required; and
- c) animal food supply should comprise all required nutrients unless the requirements of the study precludes it.

Table O.6 — Score sheet for infectious disease research

Experiment No.:		Animal No.:					
Weight (g)							
	Date/time						
APPEARANCE	Score						
Normal							
Diminished grooming							
Piloerection, discharge nose/eyes							
Soiled, poorly groomed coat							
BODY WEIGHT							
Normal < 5 %							
Body weight 5 % to 10 %							
Body weight 11 % to 15 %							
Body weight 16 % to 20 %							
CLINICAL SIGNS							
Food and water intake							
Stool normal – slightly soft							
Diarrhoea ^a							
Increased abdominal dimension, soft on palpation, no stool							
RESPONSE TO HANDLING							
Normal							
Slightly decreased or increased response							
Strongly decreased or increased response/vocalization on abdominal palpation							
Decreased or increased response							
TOTAL							
SIGNATURE							
^a 0= normal; 1 = loose faeces on the floor; 2 = pools of faeces on the floor; 3 = running out on handling. nd = not determined.							

O.11.5.2 Assessment and humane endpoints and actions

O.11.5.2.1 The following assessment ratings apply:

- a) 0 to 4: Normal.
- b) 5 to 9: Increase frequency of assessment and observe the animal more closely.
- c) 10 to 15: Clear distress present. Treat the animal if possible. Increase the frequency of observation. Consult with principal researcher or veterinarian or head animal technologist. Consider putting the animal to death by recognized euthanasia methods.

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O.11.5.2.2 If more than one (negative) clinical sign occurs then the veterinarian and the principal scientist should be informed.

Annex P (informative)

Animal welfare incident report forms

P.1 Purpose

The animal welfare incident report (see table P.1) provides a mechanism whereby person(s), who have identified animal welfare incidents related to animal research procedures, treatments, and the care and wellbeing of laboratory animals, can record important data.

This data shall be recorded, referenced and filed with the relevant Protocol Case History.

This data may be used as evidence in any animal welfare investigation, allegations of research misconduct, disciplinary hearings or applications for research funding.

P.2 Checklist for animal welfare incident report

The following list provides key indicators to aid in the completion of the incident report form.

- Animal care and wellbeing.
- Animal facility (authorized and controlled access).
- Animal handling.
- Animal monitoring/duty rosters/public holidays/weekends/after hours.
- Biohazards /noxious substances/ionising radiation/chemicals.
- Cage records/case history.
- Caging.
- Emergency procedures/contingency plans.
- Escaped animals.
- Feed and water.
- Housing.
- Photographic or video evidence.
- Post-surgical procedures/anaesthesia.
- Quarantine animals.
- Relevant and existing standard operating procedures/work instructions.
- Sanitation standards/hygiene standards.
- Staff training and expertise.
- Surgical procedures/anaesthesia.
- Transport facilities/methods/emergency kit.
- Veterinary care.

P.3 References

See bibliography.

Table P.1 — Example of an animal welfare incident report

For office use only			
Incident report reference No.	Date received	AEC protocol No.	Principal researcher/researcher
			Department
Date of incident:			
Time of incident:			
Location of incident:			
Number of animals affected:			
Animal(s) identification or description:			
Incident identified by (name, qualifications, contact details and signature):			
Incident witnessed by (name, qualifications, contact details and signature):			
Incident reported to (date, name, qualifications and contact details):			
Description of incident and observations (attach detailed statement if necessary):			

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Table P.1 (*concluded*)

Has immediate corrective action been taken?		
Yes	No	Unknown
Specify details (if known):		
Has the institutional (or other) veterinarian been contacted?		
Yes	No	Unknown
Specify details (if known):		
Researcher(s), co-worker(s) and staff responsible for animal procedures and care (specify if known):		
Research protocol number (if known):		
Department (if known):		
Additional comments or remarks:		
Additional statement(s) attached :	Yes	No
Submit report to the institutional veterinarian or chairman of the AEC.		

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