ISBN 978-0-626-39437-0

SANS 10386:2021

Edition 2

SOUTH AFRICAN NATIONAL STANDARD

The care and use of animals for scientific purposes

WARNING
This document references other documents normatively.

Published by the South African Bureau of Standards
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SANS 10386:2021
Edition 2

Table of changes

Change No.	Date	Scope

Acknowledgement

This South African standard is based on the Australian Code for the care and use of animals for scientific purposes, 8th Edition:2013, 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 prepared by National Committee SABS/TC 1040/SC04, *Nature Conservation – The care and use of animals for scientific purposes*, in accordance with procedures of the South African Bureau of Standards, in compliance with annex 3 of the WTO/TBT agreement.

This document was approved for publication in October 2021.

This document supersedes SANS 10386:2008 (edition 1).

Compliance with this document cannot confer immunity from legal obligations.

Reference is made in the note to 3.32 to the "relevant national department" for the release of animals. In South Africa this means the Department of Agriculture, Forestry and Fisheries (DAFF).

Reference is made in the note to 3.35 to the "relevant national council" for the registration of research animal facilities. In South Africa this means the South African Veterinary Council (SAVC).

Reference is made in 3.44 to the “relevant national legislation” for the para-veterinary definition. In South Africa this means the Veterinary and Para-veterinary Professions Act, 1982 (Act No. 19 of 1982).

Reference is made in 5.2.3.1.2(f) to the "relevant national legislation". In South Africa this means the National Health Act, 2003 (Act No. 61 of 2003).

Reference is made in 5.2.3.1.2((i) to the "relevant national authority". In South Africa this means the National Health Research Ethics Council (NHREC).

Reference is made in 5.3.3.2.1.2(a) to the "relevant national council" for the registration/authorization of veterinarians. In South Africa this means the South African Veterinary Council (SAVC).

Reference is made in 5.3.3.4(d), 5.5.3.1.2, 5.10.3.3.4, 8.3.1.1(d), 10.3.1, 10.3.6, 13.3.1(a) to "relevant national legislation" on the use of animals for scientific studies and teaching activities. In South Africa this means the following:

a) the Animals Protection Act, 1962 (Act No. 71 of 1962);

b) the Animal Diseases Act, 1984 (Act No. 35 of 1984);

c) the Veterinary and Para-veterinary Professions Act, 1982 (Act No. 19 of 1982);

Foreword *(continued)*

d) the Animal Health Act, 2002 (Act No. 7 of 2002);

e) the Medicines and Related Substances Control Act, 1965 (Act No. 101 of 1965); and

f) the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act No. 36 of 1947).

Reference is made in 5.3.3.4(d) and 5.5.3.2.3(s) to the "relevant national legislation" on personnel authorizations for veterinary or para-veterinary procedures. In South Africa this means the Veterinary and Para-veterinary Professions Act, 1982 (Act No. 19 of 1982).

Reference is made in 5.3.3.6.3(k) to the "relevant national departments" for the certification of animals. In South Africa this means the national or provincial (or both) Department of Agriculture, Forestry and Fisheries (DAFF) and Department of Environmental Affairs (DEA).

Reference is made in 5.3.3.6.3(l) to the "relevant national council" for the authorization of personnel. In South Africa this means the South African Veterinary Council (SAVC).

Reference is made in 5.4.3.1.2(k) to the "relevant national legislation" for registration. In South Africa this means the National Health Act, 2003 (Act No. 61 of 2003).

Reference is made in 5.6.4.2(i) to the "relevant national and provincial legislation" for obtaining special permits. In South Africa this means the Department of Agriculture, Forestry and Fisheries (DAFF).

Reference is made in 5.8.3.2(r) to the "relevant national council" for authorization of people in all procedures. In South Africa this means the South African Veterinary Council (SAVC).

Reference is made in 5.8.3.2(x) to the "relevant national legislation" for permission of using scheduled medicines. In South Africa this means the Medicines and Related Substances Act, 1965 (Act No. 101 of 1965).

Reference is made in 5.9.3.3 to the "relevant national legislation" for the overseeing of qualified individuals by the attending veterinarian. In South Africa this means the Veterinary and Para-veterinary Professions Act, 1982 (Act No. 19 of 1982).

Reference is made in 5.9.4.1(m)(4) and 6.4.20.2(b), to the "relevant national legislation" for the re-homing of animals and the related aspects to be taken into account. In South Africa this means the Animals Protection Act, 1962 (Act No. 71 of 1962).

Reference is made in 5.10.1 to the "relevant national council" on authorization/registration of performing procedures or working with or on animals remains. In South Africa this means the South African Veterinary Council (SAVC).

Reference is made in 6.3.3.1.6, 6.3.3.2.3 and 6.3.3.3.6 to the "relevant national legislation" for the registration of a competent person. In South Africa this means the Veterinary and Para-veterinary Professions Act, 1982 (Act No. 19 of 1982).

Reference is made in 6.3.3.3.1(a) "relevant national legislation" for normal behaviour and signs of pain, suffering, distress and lasting harm for the species. In South Africa this means the Veterinary and Para-veterinary Professions Act, 1982 (Act No. 19 of 1982).

Reference is made in 6.4.2(f) to the "relevant national legislation" for the carrying out of veterinary procedures. In South Africa this means the Veterinary and Para-veterinary Professions Act, 1982 (Act No. 19 of 1982).

Reference is made in 6.4.4.1(f) to the "relevant national legislation" for the identification of animals. In South Africa this means the Animal Identification Act, 2002 (Act No. 6 of 2002).

Foreword *(continued)*

Reference is made in 6.4.6.2(c) to the "relevant national legislation" for the administration of anaesthesia, analgesia and sedation, and management of pain, suffering, distress and lasting harm. In South Africa this means the Veterinary and Para-veterinary Professions Act, 1982 (Act No. 19 of 1982).

Reference is made in 6.4.17.1 to the "relevant national legislation" on introduction of foreign DNA into mammalian cells or whole animals. In South Africa this means the Genetically Modified Act, 1997 (Act No. 15 of 1997).

Reference is made in 7.3.3.6(e) and I.6.3 to the "relevant regulatory authorities" for the re-homing of animals and the related aspects to be taken into account. In South Africa this means the Department of Agriculture, Forestry and Fisheries (DAFF) and Provincial Nature Conservation Departments.

Reference is made in 7.3.4.3(a) to the "regulatory body" for registration of animal premises. In South Africa this means the South African Veterinary Council (SAVC).

Reference is made in 8.3.1.1(d), 8.3.3.2.1 and 8.3.3.2.2 to "relevant regulatory authorities" on reporting of non-compliance and breaches. In South Africa this means the Department of Agriculture, Forestry and Fisheries (DAFF) and the Department of Health.

Reference is made in 10.2.1 and 10.3.6 to the "relevant regulatory authority" on obtaining permits for the transportation of animals. In South Africa this means the Department of Agriculture, Forestry and Fisheries (DAFF), or Provincial Nature Conservation Departments (or both).

Reference is made in 10.5.1(a) to the "relevant national council" on registration and authorization of personnel. In South Africa this means the South African Veterinary Council (SAVC).

Reference is made in 10.6.1.4.5 and A.2.2(f) to the "relevant national legislation" on storage areas for flammable and controlled substances. In South Africa this means the Occupational Health and Safety Act, 1993 (Act No. 85 of 1993) and the Medicines and Related Substances Act, 1965 (Act No. 101 of 1965).

Reference is made in 11.3.1 to the "relevant national regulation" on the listing of Threatened and Endangered species. In South Africa this means the National Environmental Management: Biodiversity Act, 2004 (Act No. 10 of 2004) on Threatened or Protected Species Regulations.

Reference is made in 11.4.3(b) to the "relevant national department" on scheduled substances. In South Africa this means the Department of Health.

Reference is made in 11.5.5 to the "relevant national legislation" on the selling, inspection and laying of traps and other devices for the purposes of capturing and destroying animals. In South Africa this means the Animals Protection Act 1962, (Act No. 71 of 1962.G.19.9.1).

Reference is made in 11.6.1.2(f) to the "relevant national legislation" on use of tranquillizers or short-acting anaesthetics for capturing and destroying animals. In South Africa this means the Medicine and Related Substance Act 1965, (Act No. 101 of 1965).

Reference is made in and 12.2.3(d) to the "relevant regulatory body" on registration of teacher or demonstrator. In South Africa this means the South African Veterinary Council (SAVC), the Health Professions Council of South Africa (HPCSA) or the South African Council for Natural Science Professions (SACNASP).

Reference is made in G.12.2.1 and H.5.16 to the "relevant national department" for waste disposal. In South Africa this means the Department of Environmental Affairs (DEA).

Reference is made in G.19.7.1 and I.6.1 to the "relevant national legislation". In South Africa this means the Veterinary and Para-veterinary Professions Act, 1982 (Act No. 19 of 1982).

Foreword *(concluded)*

Reference is made in H.7.1.2 to the "relevant national departments". In South Africa this means the Marine and Coastal Management of the Department of Environmental Affairs (DEA) and the Department of Agriculture, Forestry and Fisheries (DAFF).

Reference is made in H.12.5.3 to the "relevant regulations". In South Africa this means the Nature and Environmental Conservation Regulations.

Reference is made in annex R to the “relevant national legislation”. In South Africa this means the Department of Agriculture, Forestry and Fisheries (DAFF) in terms of the Animal Diseases Act, 1984 (Act No. 35 of 1984).

Reference is made in S.4.1 to the "relevant national department" for obtaining special permits. In South Africa this means the Department of Agriculture, Forestry and Fisheries (DAFF).

Reference is made in the bibliography (References – Animal welfare incident report forms) to the relevant national legislation. In South Africa this means the Animal Protection Act, 1962 (Act No. 71 of 1962).

Reference is made in the bibliography (References – Animal welfare incident report forms) to the relevant national legislation. In South Africa this means the Veterinary and Para-veterinary Professions Act, 1982 (Act No. 19 of 1982).

Annex A forms an integral part of this document. Annexes B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S and T are for information only.

Introduction

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

a) emphasize the responsibilities of the researchers, the teachers, the animal care personnel and the institutions using and caring for animals,

b) ensure that the welfare of animals is always maintained in accordance with this standard,

c) promote the implementation of the principles of the four Rs, that is to Replace, Reduce, Refine and to take Responsibility for animal use in scientific studies and teaching activities,

d) ensure that the use of animals is justified by the establishment of functional and properly constituted Animal Ethics Committees (AECs) to ensure adherence to the principles of the four Rs,

e) ensure that the use of animals for scientific purposes shall have scientific or educational merit; shall aim to benefit humans, animals or the environment; and are conducted with integrity,

f) prevent or minimize pain, suffering, distress or lasting harm (or both), where possible, for each animal used in scientific studies and teaching activities,

g) ensure minimum uniform national standards for the use and care of animals for scientific and teaching purposes in accordance with annex A (refer to annexes B to T),

h) ensure that the use of animals for scientific or educational (or both) purposes is only considered where a non-animal alternative is not available, and

i) ensure that all personnel who perform procedures, supervise procedures or care for animals are adequately educated, trained and confirmed competent.

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The care and use of animals for scientific purposes

1 Scope

1.1 This standard encompasses all aspects of the care and use of, or interaction with, animals for any scientific purposes including but not limited to medicine, biology, agriculture, wildlife, veterinary, conservation, physiology, zoology and other animal sciences, as well as industry and teaching studies in South Africa. It includes animal use in research, teaching, training, field trials, product testing, diagnosis, the production of biological substances or responses and environmental studies. This standard is applicable to all research teaching and use of animals for scientific purposes by any institution or entity.

1.2 It provides general principles for the care and use of animals, specifies the responsibilities of the researchers and the institutions, and details the terms of reference, membership and operation of the 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.

1.3 It covers all live non-human vertebrates and higher invertebrates such as the *Cephalopoda* and *Decapoda*. It also covers embryos, fetuses and larval forms and their treatment in a humane manner once they have progressed beyond half the gestation or incubation period of the relevant species, or they become capable of independent feeding, whichever comes first.

NOTE 1 The researchers should submit proposals to use all invertebrates for consideration by the AECs.

NOTE 2 SANS 10386 cannot be interpreted to condone studies that are considered morally wrong or offensive. South African legislation and institutional policies govern such instances.

2 Normative references

The following referenced documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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 South African Bureau of Standards.

2.1 Standards

SANS 994-1, *Ratite farming – Part 1: Ostriches*.

SANS 1478, *Pig welfare*.

SANS 10379, *Zoo and aquarium practices*.

2.2 Other publications

AAALAC International. AAALAC International Position Statement on The Attending veterinarian and Veterinary Care [Internet]. Available from: https://www.aaalac.org/accreditation/positionstatements.cfm#vetcare.

ARP 1048, *Wildlife rehabilitation – General considerations*.

Department of Health. *Ethics in Health Research: Principles, Structures and Processes.* Pretoria: DOH, 2015.

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

National Health and Medical Research Council (NHMRC). 2007. *Guidelines for the generation, breeding, care and use of genetically modified and cloned animals for scientific purposes.*

Fowler, M. E. 2011. *Restraint and handling of wild and domestic animals*. John Wiley & Sons.

International standards: the World Organisation for Animal Health (OIE). 2011. *Terrestrial Animal Health Code.*

3 Definitions

For the purposes of this document, the following terms and definitions apply.

3.1
adverse event
any event that has a negative impact on the well-being of an animal

3.2
alternatives
refers to the replacement of the use of animals either by methods that do not involve animals at all (absolute replacement) or by those that use only the cells or tissues of animals killed for that purpose (relative replacement)

NOTE Alternatives may also refer to alternative experimental design or statistical techniques that reduce the number of animals used or to alternative methods that lead to a refinement for animals that do undergo testing.

3.3
animal
live, non-human vertebrate, including fertilized eggs, fetuses and embryos, i.e. fish, amphibians, reptiles, birds and mammals, and encompassing domestic animals, feral animals, purpose-bred animals, farm animals, wildlife and higher invertebrates, such as the *Cephalopoda* and *Decapoda* (for example, octopus, squid, cuttlefish)

NOTE Refer also to 3.19 and 3.26.

3.4
animal caretaker
any person involved in the care of an animal (basic, technical or veterinary care) including during the acquisition, transport, breeding, housing, husbandry, treatment, experimentation or disposal of the animal, and this will include veterinary para-professionals

3.5
Animal Ethics Committee
AEC
South African committee constituted in accordance with the terms of reference, operating procedures and membership laid down in this standard (see 5.3)

3.6
animal studies
conducting scientific activities that use animals for any of the following purposes:

a) to advance knowledge;

b) to test a hypothesis;

c) to supply a product ;

d) to produce a biological substance;

e) to provide organs, tissues, cells, gametes, biological substances including blood or blood products or serum, eggs, embryos or fetuses;

f) to act as a host;

g) to impart or demonstrate existing knowledge;

h) to teach or learn surgical and other procedures, techniques or methods, both invasive and non-invasive including behavioural experiments;

i) to test or collect data on any substance or product, including to comply with statutory requirements; and

j) to conduct observational studies including to make audio or visual recordings.

3.7
competence
consistent application of knowledge and skill to the standard of performance required regarding the care and use of animals

3.8
direct supervision
carry out instructions in the supervisor’s presence, provided that the supervisor gives the person and the animal, his or her undivided attention

3.9
distress
aversive negative state in which coping and adaptation processes fail to return an animal to physical and physiological homeostasis

3.10
domestic animal
animal that has been domesticated by humans so as to live and breed in a tame condition and to depend on human kind for survival

3.11
environmental enrichment
process of providing stimulating environments for animals to demonstrate species typical behaviour which allows them to exercise control or choice over their environment, and to enhance their well-being which includes measures taken to alleviate boredom and to eliminate abnormal or harmful behaviour

3.12
euthanasia
act or practice of ending the life of an animal as painlessly and humanely as possible

3.13
facilities
refer to 3.35

3.14
farm animals
animals that are used for the production of human and animal food and feed, fibre, skin and hide and are being used in farm work, including but not limited to cattle, sheep, goats, alpacas, pigs, poultry, ostriches, horses, fish, crocodiles, rabbits and wildlife i.e. intensively farmed

3.15
feral animal
domestic animal that exists in a wild state

3.16
humane endpoint
describes a point before the designed experimental endpoint where the animal should be removed from the study, upon the commencement of an animal exhibiting physiological or behavioural signs (or both) indicating that a predetermined level of stress, anxiety, pain, or physical damage or other kind of harm has been reached

NOTE When the relevant criteria is identified, actions such as euthanasia, removal of the animal, modification of the experimental design, analgesic administration or treatment of the animals (or both) can be carried out to prevent or alleviate pain or distress (or both) regardless of whether the aims of the study have been achieved. In some cases, studies may result in severe or chronic pain or significant alterations in the animals' ability to maintain normal physiology, or adequately respond to stressors and should include descriptions of appropriate humane endpoints or provide science-based justification for not using a particular, commonly accepted humane endpoint.

3.17
institution
any organization or agency involved in the care and use of animals for scientific purposes, including universities, hospitals, research institutes, government departments, teaching organizations (including schools and colleges), vocational training organizations, agricultural organizations, commercial companies, and organizations involved in animal breeding and supply

3.18
Institutional Biological Safety Committee
IBSC
committee charged with reviewing the proposed use of biohazardous agents, nano-materials, human material, and recombinant deoxyribonucleic acid (DNA) molecules to assess compliance with applicable regulatory guidelines

3.19
laboratory animal
animal (refer to 3.3) kept and used for scientific purposes within the confinement of secure physical boundaries of a research animal facility

3.20
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 It encompasses the study of the biology of laboratory animals, their husbandry and environmental requirements, genetic and microbiological standardization procedures, prevention and treatment of diseases, optimization of the study of techniques and the improvement of anaesthesia, analgesia and euthanasia.

3.21
moribund
being in the state of dying, or approaching death with no chance of recovery

3.22
pain
unpleasant sensory and emotional experience associated with actual or potential tissue damage, occurring in varying degrees of severity, for example, as a consequence of a bodily disorder such as induced or accidental injury, confinement, disease

3.23
partial replacement
where alternative method(s) is not capable of fully replacing the existing animal model for a procedure for example, making use of cultured animal cell lines or tissues taken from animals killed solely for this purpose (i.e. not having being subject to a procedure)

3.24
person-in-charge
person who, by appointment or delegation, is suitably qualified and competent to be in charge of a research animal facility, with total responsibility to ensure the care and well-being of the animals in that facility

3.25
pest
any living organism which has a harmful effect on humans, the environment or other animals (their food or their living conditions)

3.26
privately owned animal
animal that is used for scientific purposes with consent of the owner outside the physical boundaries of a research animal facility and may be housed on a farm, a small holding or in an urban household

NOTE With these animals being privately owned, the researcher or the research institution is not the legal owner of these animals (refer to 3.3 for the definition of an animal).

3.27
project
any scientific study or teaching and training activities that has been approved by the AEC

3.28
proposal
formal written outline of a scientific study or teaching and training activities submitted to the AEC for consideration and approval

3.29
reduction
methods that minimize the number of animals used per experiment or study, by enabling the researchers to obtain comparable levels of information from fewer animals or more information from the same number of animals, thereby avoiding further animal use

3.30
refinement
methods that minimize the pain, suffering, distress or lasting harm which may be experienced by the animals

3.31
regulatory
serving or intended to regulate by control or discretion in accordance with the rule, the principle or the law

3.32
rehabilitation
restoring an animal to a condition of good physical and psychological health, which may include preparation for release to the appropriate environment

NOTE Release of animals may be subject to approval by the relevant national department (see foreword).

3.33
replacement
methods that avoid or replace the use of animals in an experiment where they would have otherwise been used

3.34
researcher
person who is recognized by the AEC as competent (including qualification, training, experience and practical skills) to conduct an approved scientific research study, which involves animals, including but not limited to in research animal facilities, approved areas of captivity, or the natural environment

3.35
research animal facility
any facility or area, where animals may be used, maintained or bred for scientific purposes, including for research, testing, teaching, training, validation, production or observation

NOTE Relevant research animal facilities should be registered with the relevant national council (see foreword).

3.36
scientific study
study or a series of related studies that form a defined 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, through organized and systematic investigation

3.37
scientific purposes
use of animals in any animal study (see 3.6) including but not limited to the use for research, testing, teaching, training, validation, production or observation

3.38
sentient
having the power of perception by the senses, the capacity to feel, perceive or experience subjectively and it implies the ability to experience pleasure and pain (for example, to suffer)

NOTE This standard recognizes that animals are sentient.

3.39
stereotypic behaviour
repetitive sequence of movements without an apparent goal, which are derived from normal maintenance behaviour but appear out of context, exaggerated or unusually sustained, often due to lack of environmental enrichment

NOTE Stereotypies and self-harm behaviours are repetitive or harmful behaviours or postures only observed in captivity (or all of these), often arising in response to an abnormal environment (for example, a lack of choice, control, and opportunity to express species-specific behaviours. These behaviours are signs of negative well-being, as they indicate an attempt to cope with chronic stress and may be accompanied by physiological changes).

3.40
stress
real or perceived challenge to an animal's physiological homeostasis or psychological well-being

3.41
teacher
person who is recognized by the AEC as competent to conduct an approved teaching or training activity (or both) which involves animals including but not limited to in research animal facilities, approved areas of captivity, or the natural environment

3.42
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

3.43
unscheduled
unplanned event that occurs to an animal that was not predicted in the project or expected for the animal

3.44
veterinary para-professional
professions defined in the relevant legislation (see foreword)

NOTE Veterinary para-professional professions render services supplementing veterinary service including animal health technicians, laboratory animal technologists, veterinary nurses and veterinary technologists.

3.45
welfare
state of the well-being brought about by meeting the physical, environmental, nutritional, behavioural and social needs of the animal

3.46
well-being
animal that is in a positive mental state and is able to achieve successful biological function, to have positive experiences, to express innate behaviours, and to respond to and cope with potentially adverse conditions

NOTE An animal well-being may be assessed by physiological and behavioural measures of an animal's physical, clinical and psychological health and of the animal's capacity to cope with stressors, and species-specific behaviours in response to social and environmental conditions.

3.47
wildlife
indigenous or non-indigenous species of animals that are not covered under the definition of farm or domestic animal (see 3.10 and 3.14)

3.48
xenotransplantation
transplantation of living cells, tissues or organs from one species to another

NOTE A "recipient animal" is an animal that receives a transplant, implant or infusion of either live cells, tissues or organs from another species, or body fluids, cells, tissues or organs that have ex vivo contact with live cells, tissues or organs from another species. A "source animal" is an animal from which body fluids, cells, tissues or organs for use in xenotransplantation are obtained.

3.49
xenosis
potential spread of pathogens from a source animal of one species to a recipient animal of another species and, potentially, to the general population of the recipient species

4 Governing principles in the care and use of animals for scientific purposes

4.1 General

This clause describes the governing principles and the ethical framework that serves to guide decisions and actions of all people involved in the care and use of animals for scientific purposes. The application of these governing principles is further developed in subsequent clauses of this standard. Each person involved in the care and use of animals for scientific purposes shall consider the governing principles when applying this standard to their specific circumstance.

4.2 Governing principles

4.2.1 Respect for animals shall underpin all decisions and actions of all people involved in the care and use of animals for scientific purposes. This respect is demonstrated by

a) using animals only when it is justified,

b) supporting the well-being of the animals involved,

c) avoiding or minimizing harm, including pain, suffering, distress and lasting harm, to those animals,

d) applying high standards of scientific integrity,

e) applying the four Rs at all stages of animal care and use:

1) the *Replacement* of animals with alternatives;

2) the *Reduction* in the number of animals used;

3) the *Refinement* of techniques used to minimize the adverse impact on animals; and

4) the knowledge and acceptance of one's *Responsibilities*.

4.2.2 The care and use of animals for scientific purposes shall be subject to ethical review. The AEC shall be satisfied that there is sufficient evidence to support a case that the proposed use of animals is justified.

4.2.3 A judgement as to whether a proposed use of animals is ethically acceptable shall be based on information that demonstrates the governing principles in 4.2.1. This judgement shall balance whether the potential negative effects on the well-being of the animals involved is justified by the potential benefits.

4.2.4 The obligation to respect and preserve the dignity of animals shall always prevail. The responsibilities associated with this obligation, apply throughout the animal's lifetime, including acquisition, transport, breeding, housing, husbandry, use of the animal in a project, and provisions for the animal at the conclusion of their use.

4.3 Justification of the use of animals

4.3.1 Evidence to support a case to use animals shall demonstrate that

a) a project has scientific or educational merit, and has the potential benefit for humans, animals or the environment,

b) the use of animals is essential to achieve the stated aims, and suitable alternatives to replace the use of animals to achieve the stated aims are not available,

c) the project involves the minimum number of animals required to obtain valid data, and

d) the project involves the minimum adverse impact on the well-being of the animals involved.

4.3.2 Projects shall only be undertaken

a) to obtain and establish significant information relevant to the understanding of humans or animals (or both),

b) to maintain and improve human or animal (or both) health and welfare,

c) to improve animal management or production,

d) to obtain and establish significant information relevant to the understanding, maintenance or improvement of the natural environment, or

e) to achieve educational outcomes in science, as specified in the relevant curriculum or competency requirements.

4.4 Support the well-being of animals

4.4.1 The well-being of animals used for scientific purposes shall be considered in terms of the cumulative effects of an animal's lifetime experience. At all stages of the care and use of an animal, measures should be taken to ensure that the animal's environment and management are appropriate for the species and the individual animal, and support the animal's well-being.

4.4.2 Practices and procedures used for the care and management of animals shall be based on current best practice that

a) takes into consideration the relevant aspects of species-specific biology, physiology and behaviour,

b) is based on the best available scientific evidence (or, in the absence of scientific evidence, accepted practice), which includes the potential adverse impact of conditions and procedures on the well-being of the animals, or

c) includes strategies to minimize adverse impacts.

4.4.3 Special ethical consideration and AEC approval are required where the conditions specified in 4.4.2 are precluded by the requirements of a project or activity.

4.5 Avoid or minimize harm, including pain, suffering, distress and lasting harm to animals

4.5.1 Animals have a capacity to experience pain, suffering, distress and lasting harm, even though they may perceive and respond to circumstances differently from humans. Pain, suffering, distress and lasting harm may be difficult to evaluate in animals. Unless there is evidence to the contrary, it shall be assumed that the procedures and the conditions that would cause pain, suffering, distress and lasting harm in humans or other animals cause pain, suffering, distress and lasting harm in animals. Decisions regarding the possible impact of the procedures or the conditions on an animal's well-being shall be made in consideration of an animal's capacity to experience pain, suffering, distress and lasting harm.

4.5.2 All possible steps shall be taken at all times to safeguard the well-being of animals by avoiding or minimizing harm, including pain, suffering, distress and lasting harm to the animals.

4.5.3 In the cases where the inclusion of analgesic support could be deemed to interfere with the study, the researcher shall undertake a pilot study to demonstrate whether the said medication interferes with the study outcome.

NOTE Comprehensive literature review should be done to demonstrate why an analgesic could interfere physiologically, as available.

4.5.4 Where the aim(s) of the project involves the animals experiencing pain, suffering, distress and lasting harm that will not be alleviated, the planned endpoint of the project shall be as early as feasible to avoid or minimize pain, suffering, distress and lasting harm in the animals.

4.5.5 Death as an endpoint shall be avoided unless it is essential for the aim(s) of the project. In these circumstances, the means to prevent or minimize harm, including pain, suffering, distress and lasting harm, shall be considered, implemented and reviewed at all stages of the project. Sound scientific evidence shall be provided for using death as an endpoint, for example, conducting a relevant pilot study. Where possible, it should be substituted by more humane endpoints using clinical signs that determine the impending death, thereby allowing the animal to be killed without any further suffering. Where death as the endpoint is unavoidable, the procedure shall be designed so as to

a have few animal deaths as possible, and

b) reduce the duration and intensity of suffering to the animal to the minimum possible and, as far as possible, ensure a painless death.

4.5.6 Prompt action shall be taken to alleviate pain, suffering, distress and lasting harm that were not anticipated in an approved project or activity, or occur as the result of an emergency. Such action shall take precedence over an individual animal reaching the planned endpoint of the project or activity, or the continuation or completion of the project or activity.

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

4.6 Apply high standards of scientific integrity

4.6.1 Regardless of the potential benefits of a project, the methods used shall be scientifically valid, feasible, well designed and carefully conducted so that there is a reasonable expectation that the aims of the project will be achieved. Projects that are not scientifically valid shall not be performed, no matter how mild the impact on the well-being of the animals. For training projects that involve animals, the training shall be applicable to the needs of the country as well as ethically justifiable.

4.6.2 The investigators shall use methods that are in accordance with the current best practice that

a) take into consideration the relevant aspects of species-specific biology, physiology and behaviour,

b) are based on the best available scientific evidence, which includes the potential adverse impact of conditions and procedures on the well-being of the animals, and

c) include strategies to minimize adverse impacts.

4.6.3 Animals that are used shall be suited for the purpose of the project or activity, taking into account their biological characteristics, including morphology, physiology, behaviour, genetic make-up, temperament and behavioural conditioning, microbiological and nutritional status, and general state of health.

4.7 Apply replacement, reduction, refinement and responsibilities (the four Rs) at all stages

4.7.1 Replacement

4.7.1.1 Methods that promote the replacement or partial replacement (for example, ex-vivo primary cell cultures) of the use of animals shall be investigated, considered and, where applicable, implemented.

4.7.1.2 Before the use of animals is considered, all existing information relevant to the proposed aim(s), including existing databases, shall be examined. Replacement techniques that shall be considered include, but are not limited to

a) the use of epidemiological data,

b) physical and chemical analysis,

c) computer,

d) mathematical and inanimate synthetic models,

e) simulations,

f) in vitro systems,

g) non-sentient organisms,

h) cadavers, and

i) clinical cases.

4.7.1.3 Opportunities to replace the use of animals shall be kept under review during the lifetime of a project. Where relevant and applicable, the outcome of this review shall be implemented in current projects and taken into account in planning future projects.

4.7.2 Reduction

4.7.2.1 The number of animals used in a project shall be the minimum necessary to achieve the proposed aim(s) and to satisfy good statistical design. The use of too few animals may invalidate the experimental result and result in wastage of animals.

4.7.2.2 The number of animals used may be reduced by the appropriate reuse of individual animals. The benefits of reusing animals shall be balanced against any adverse effects on their well-being, taking into account the lifetime experience of the individual animal. Reuse of animals requires particular justification and specific AEC approval.

4.7.2.3 Activities involving the use of animals shall not be repeated within a project or between projects unless such repetition is essential for the purpose or design of the project (for example, sound experimental design, statistical analysis, corroboration by the same or another investigator).

4.7.2.4 As a guiding principle, reducing the number of animals used should not result in greater harm, including pain, suffering, distress and lasting harm, to the individual animals used. The benefits of reducing the number of animals shall be balanced against any adverse effects on their well-being, taking into account the lifetime experience of the individual animal. This requires particular justification and specific AEC approval.

4.7.2.5 All possible steps shall be taken to reduce factors that are not part of the experimental design of the project and are known to contribute to variability of experimental results, including the use of animals of known genetic, biological and behavioural background, standardized environmental conditions etc. Reduction of experimental variables may result in reduced animal use.

4.7.2.6 Where practicable, tissue and other biological material from animals being killed shall be shared amongst the investigators or deposited into a tissue bank for subsequent distribution.

4.7.2.7 Breeding of animals shall be managed to avoid or minimize the production of excess animals. A new line of animal should not be generated if a similar suitable animal line is available to the investigator. When a new animal line is generated, the colony should be made available as a source for other investigators, as appropriate.

4.7.3 Refinement

4.7.3.1 Steps shall be taken at all times to support and safeguard the animal well-being. The effectiveness of strategies for supporting and safeguarding animal well-being shall be kept under review during the lifetime of activities, including projects. Where relevant and applicable, the outcome of this review shall be implemented in current activities and taken into account in planning future activities, including projects.

4.7.3.2 People who care for and use animals shall ensure that procedures are performed competently, and

a) be competent for the procedure they perform, or

b) be under the direct supervision of a person who is competent to perform the procedure.

4.7.3.3 The duration of activities shall be no longer than required to meet the aim(s) of the project, and shall be compatible with supporting and safeguarding animal well-being. Animals shall not be held for prolonged periods as part of an approved project before their use, without the AEC approval.

4.7.3.4 Animals chosen for scientific purposes shall be suitable for the purposes of the investigation, taking into account their biological characteristics, including behaviour, temperament, physiology, morphology, genetic constitution and nutritional, microbiological and general health status.

4.7.3.5 The researchers and the teachers shall use the current best practice, and shall be competent in the procedures they perform. It is incumbent on the AEC to ensure that person involved with the said activities have documented proof of competence.

4.7.3.6 The well-being of the animals shall be the primary consideration in the provision of care, and shall be based on the behavioural and biological needs of the species including housing, husbandry and appropriate environmental enrichment. Animals shall be transported, housed, fed, watered, handled, bred and used under conditions that are appropriate to the well-being of the species as well as of individual animals.

4.7.4 Accept responsibilities

4.7.4.1 The institutions, the AECs, and the people involved in any aspect of the care and use of animals for scientific purposes shall be aware of and accept their responsibilities (see clause 5), and act in accordance with this standard, and be held accountable for this.

4.7.4.2 All activities, including projects, that involve the care and use of animals for scientific purposes shall

a) be subject to ethical review, approval and monitoring by the AEC,

b) commence only after approval has been granted by the AEC,

c) be conducted in accordance with the AEC approval, and

d) cease if approval from the AEC is suspended or withdrawn or period of said approval ends.

5 Responsibilities

5.1 General

This clause describes the responsibilities of

a) the institutions (see 5.2),

b) the institutions regarding the governance of the animal ethics committee (see 5.3),

b) the animal ethics committees (see 5.4),

c) the investigators (including teachers and people involved in wildlife studies) (see 5.5),

d) the animal caretakers (see 5.6),

e) the institutions, the investigators and the AEC in situations which involve the use of external the AEC, more than one institution or animal ethics committees (or all of these), and projects conducted in other countries (see 5.7),

f) the institutions with respect to developing an application form to the AEC (see 5.8),

g) attending veterinarian and veterinary care (see 5.9), and

h) the institutions on education, training, supervision and the competency of personnel (see 5.10).

5.2 Responsibilities of the institutions

5.2.1 General

This sub-clause describes the responsibilities of the institutions regarding the care and use of animals for scientific purposes. The responsibilities of institutions regarding the governance of the AEC are described in 5.3. The responsibilities of the AECs regarding ethical review, approval and monitoring of animal care and use are described in 5.4.

5.2.2 Governing principle

Each person involved in the care and use of animals for scientific purposes shall consider the governing principles in clause 4 when applying this standard to their specific circumstance. In particular, the institutions, the AECs, and the people involved in any aspect of the care and use of animals for scientific purposes shall be aware of and accept their responsibilities, and act in accordance with this standard (see 4.7.4.1).

5.2.3 Responsibilities

5.2.3.1 General

5.2.3.1.1 The governing body of the institution is responsible for ensuring that the care and use of animals for scientific purposes conducted on behalf of the institution complies with this standard.

5.2.3.1.2 Institutions shall:

a) Ensure, through the operation of the AEC, that all activities involving the care and use of animals comply with this standard.

b) Promote compliance with this standard.

c) Ensure and support the effective operation of the AEC.

d) Identify clear lines of responsibility, communication and accountability.

e) Ensure that all people involved in the care and use of animals understand their responsibilities and the requirements of this standard, have the necessary skills and knowledge, and have access to appropriate educational programmes and resources.

f) Regularly monitor and review the institution’s compliance with this standard.

Make provision for the prompt, safe and sanitary disposal of animal carcasses and waste material in accordance with the relevant national legislation (see foreword), the local council by-laws and the community standards. Methods of disposal and storage on-site shall be approved by the institutional biosafety officer or the Institutional Biological Safety Committee (IBSC) (or both), and shall be monitored by the person-in-charge of the facility. SOPs (see 5.3.3.5.11.1) shall clearly describe these activities.

g) Ensure that the institutions are appropriately registered.

h) Ensure a safe-working environment with appropriate management of hazards including biohazards. The institution shall ensure that the advice of the institution’s biohazards (or comparable entity) committee has been sought, and that appropriate measures for containment, disposal and decontamination have been established.

i) Ensure that the AECs evaluate applications relating to health research, as defined in the relevant national legislation (see foreword) and the *Ethics in Health Research: Principles, Structures and Processes*. The AECs require registration with the relevant authority (see foreword).

j) Ensure that all records are kept for at least five years.

5.2.3.2 Ensure compliance through the AEC

5.2.3.2.1 The institutions shall ensure, through the operation of the AEC, that it is constituted and functioning in accordance with 5.3 and 5.4, and directly responsible to the governing body of the institution, and that all activities involving the care and use of animals comply with this standard.

5.2.3.2.2 The institution may use the external AEC or share the AEC with another institution (see 5.7.3.2). An external AEC is an AEC that is not part of the institution, but fulfils the function of the AEC for that institution.

5.2.3.3 Promote compliance

The institutions shall promote compliance with this standard by:

a) Nominating a senior member of the institution who will be responsible for the overall institutional governance with respect to the care and use of animals.

b) Providing adequate resources to ensure that the AEC and the people involved in the care and use of animals can meet their responsibilities, including monitoring animals and managing adverse impacts on their well-being.

c) Promoting and facilitating adoption of the governing principles of this standard in all aspects of the animal care and use, including coordinating planning and operations, and sharing resources and information, and facilitating the application of the four Rs.

d) Ensuring that policies and procedures are made available to all the relevant people and the AEC members, and are promoted within the institution. This includes institutional policies on the care and use of animals, work health and safety, confidentiality, freedom of information legislation, legal requirements, conscientious objection in the case of teaching activities, privacy and commercial-in-confidence considerations.

e) Ensuring that guidelines for animal care and use are developed in consultation with the AEC, approved by the AEC, and implemented and promoted within the institution. Guidelines should include:

   1) How the competency of people involved in the care and use of animals will be assessed and ensured.

   2) Strategies to ensure the maintenance of a health status of the animals that safeguards animal well-being and meets the requirements of their proposed use.

   3) Monitoring and assessment of animals to ensure that any harm, including pain and distress, is promptly detected and managed.

   4) Actions required for unscheduled adverse events and emergencies, including those that require welfare interventions such as the emergency treatment or humane killing of any animal, to ensure that adverse impacts on animal well-being are addressed rapidly. Such guidance should include timeframes for actions, prompt reporting to the AEC, liaison between the animal carers and the investigators, and circumstances when consultation with the

veterinarian, the performance of a necropsy by a competent person, and access to diagnostic investigations are required.

5) Approval, in advance, for the immediate use of animals, if required, for the diagnosis of unexplained and severe disease outbreaks, or morbidity or mortality, in animals or people.

f) Ensuring availability and access to veterinary advice for the management and oversight of a programme covering veterinary care, quality management and project design to safeguard the animal well-being.

g) Considering the appointment of an officer with veterinary or other appropriate qualifications, who is authorized by the institution to ensure that activities proceed in compliance with this standard and the decisions of the AEC.

5.2.3.4 Ensure and support the effective operation of the AEC

The institutions shall ensure and support the effective operation of the AEC by

a) implementing policies and procedures so that the care and use of animals is ethically reviewed, approved and monitored by the AEC,

b) ensuring that the terms of reference of the AEC are publicly available,

c) providing mechanisms for prompt and effective response to recommendations from the AEC to ensure that the care and use of animals for scientific purposes within the institution complies with this standard,

d) addressing concerns raised by the AEC regarding non-compliance with this standard that may include disciplinary action upon the advice of the AEC,

e) seeking advice from the AEC on all matters that may affect the welfare of animals used for scientific purposes by the institution, including the building or modification of animal facilities or areas adjacent to animal facilities,

f) empowering the AEC with appropriate mandate, authority and independence (including of reporting lines) to freely and responsibly review and approve or disapprove applications, and to freely and responsibly monitor, suspend or withdraw approval of approved projects, without undue interference and by ensuring proper management of any conflict of interest, and

g) providing indemnity against personal liability to all the AEC members acting in good faith.

5.2.3.5 Identify clear lines of responsibility, communication and accountability

5.2.3.5.1 Every institution should appoint the institutional official, for example, the individual who, as a representative of senior administration, bears ultimate responsibility for the animal care and use programme, and is responsible for resource planning and ensuring alignment of programme goals with the mission of the institution.

5.2.3.5.2 The institutional official should have the authority to allocate the resources needed to ensure the overall effectiveness of the programme.

5.2.3.5.3 The institutional official bears ultimate responsibility for the programme, although the overall programme direction should be a shared responsibility amongst the institutional official, the attending veterinarian, and the AEC.

5.2.3.5.4 The programme shall be clearly and regularly communicated to the institutional official by the attending veterinarian, the AEC, and others associated with the programme (for example, facilities management staff, occupational health and safety personnel, scientists).

5.2.3.5.5 The institutions shall identify clear lines of responsibility, communication and accountability by

a) Ensuring that a person is responsible for the well-being of animals at any given time and is clearly identified so that:

   1) Animal well-being is monitored by competent people at all stages and sites of animal care and use. The scope of day-to-day monitoring shall be clearly outlined and communicated to all parties.

   2) Appropriate actions are taken in cases of unscheduled adverse events and emergencies that require welfare interventions, such as treatment or humane killing of an animal.

   3) Disease outbreaks and emergencies, such as fire, power failure and biosafety issues, are promptly detected and effectively managed.

b) Ensuring that procedures are developed for addressing complaints and non-compliance relating to the care and use of animals for scientific purposes (see clause 8).

5.2.3.6 Ensure understanding of responsibilities

The institutions shall ensure that all people involved in the care and use of animals understand their responsibilities and the requirements of this standard. They shall ensure that they are competent for the procedures they perform or they are under the direct supervision of a person who is competent to perform the procedures, and have access to appropriate education programmes and resources

a) with respect to the investigators by

   1) ensuring that the investigators are well informed of their responsibilities under this standard and their legal responsibilities, and

   2) providing adequate resources for appropriate education, training and assessment of competence of the investigators, and certification of such competence to the satisfaction of the AEC.

b) with respect to animal care and management, animal carers and veterinary services by

   1) ensuring that practices and procedures for the care and management of animals are based on the current best practice,

   2) employing adequate numbers of competent people to care for animals,

   3) ensuring that the care and management of animals is under the direction of competent people with appropriate animal care or veterinary qualifications or experience, and

   4) ensuring availability and access to appropriate veterinary and diagnostic services so that a health status of the animals is maintained that safeguards animal well-being and meets the requirements of their proposed use.

c) with respect to work health and safety by

   advising the relevant personnel and the AEC members of the potential disease hazards and other occupational health and safety issues associated with the care and use of animals.

d) with respect to projects involving more than one institution or animal ethics committee (or both), and projects conducted in other countries by

   ensuring that procedures are developed in accordance with 5.7 and, that the relevant people are aware of their responsibilities in these situations.

5.2.3.7 Monitor and review compliance

5.2.3.7.1 The institutions shall regularly monitor and review institutional compliance with this standard by

a) once formally instituted within South Africa, ensuring that an independent external review is conducted at least every four years to assess the institution's compliance with this standard, and, ensuring the continued suitability, adequacy and effectiveness of its procedures to meet its responsibilities under this standard (see clause 9),

b) conducting an annual review of the operation of the AEC (see 5.3.3.1(d), 5.3.3.6 and 5.4.3.8.1 to 5.4.3.8.2 (inclusive)),

c) conducting an annual review of the effectiveness of its processes regarding complaints and non-compliance relating to the care and use of animals for scientific purposes (see clause 8).

5.2.3.7.2 The institutions should consider making publicly available

a) an annual report of compliance with this standard, and

b) a summary of the independent external review report (see clause 9).

5.3 Responsibilities of the institutions regarding the governance of the AEC

5.3.1 General

5.3.1.1 This clause describes the responsibilities of the institutions that establish the AEC regarding the governance of the AEC. The responsibilities of the AECs regarding ethical review, approval and monitoring of animal care and use are described in 5.4. The responsibilities of the institutions regarding the care and use of animals for scientific purposes are described in 5.2.

5.3.1.2 The institutions that use animals for scientific studies and teaching activities shall establish one or more AECs comprising members who are directly responsible to the governing body of the institution or its delegate. Institutions may access external AEC.

5.3.2 Governing principles

Each person involved in the care and use of animals for scientific purposes shall consider the governing principles in clause 4 when applying this standard to their specific circumstance. In particular:

a) the Institutions, the AECs and the people involved in any aspect of the care and use of animals for scientific purposes shall be aware of and accept their responsibilities, and act in accordance with this standard (see 4.7.4.1); and

b) all activities, including projects that involve the care and use of animals for scientific purposes shall

   1) be subject to ethical review, approval and monitoring by the AEC,

   2) commence only after approval has been granted by the AEC,

   3) be conducted in accordance with the AEC approval, and

   4) cease if approval from the AEC is suspended or withdrawn or period of said approval ends (see 4.7.4.2).

5.3.3 Responsibilities

5.3.3.1 General

The institutions that establish the AEC shall

a) ensure that the AEC membership will allow the committee to meet its responsibilities. Membership shall comprise at least four people, one from each of four categories of membership (see 5.3.3.2.1.2),

b) ensure that the AEC has terms of reference that are publicly available,

c) provide the AEC with the resources required to carry out its responsibilities, and to maintain the AEC,

d) establish procedures for the effective governance and operation of the AEC that will enable the AEC to meet its responsibilities under this standard and the relevant institutional policies, and promote competent and timely ethical review of animal care and use, and

e) conduct an annual review of the operation of the AEC (see 5.3.3.6).

5.3.3.2 Ensure appropriate AEC membership

5.3.3.2.1 Composition of the AEC

5.3.3.2.1.1 Chairperson

5.3.3.2.1.1.1 The institutions shall appoint the chairperson of the AEC who holds a senior position in the institution. If the chairperson is an external appointee, the institutions shall provide the chairperson with the necessary support and authority to carry out the role. The chairperson shall be appointed in addition to categories A to D members (see 5.3.3.2.1.2).

5.3.3.2.1.1.2 The institutions should consider appointing the chairperson who is independent of the care and use of animals for scientific purposes. In the cases where the independent chairperson cannot be appointed, the institution needs to put in place adequate provision for conflict of interest.

5.3.3.2.1.1.3 In addition, the chairperson should have experience in research methodology and training in animal ethics (or should have served on the AEC for a period of a year).

5.3.3.2.1.2 Members

The institutions shall ensure that membership of the AEC comprises at least one person from each of the following four categories of membership:

a) Category A – A person with qualifications in veterinary science, who is registered or authorized as a veterinarian in terms of the relevant national council (see foreword), and with experience relevant to the institution's activities or the ability to acquire relevant knowledge.

b) Category B – A suitably qualified person with substantial and recent experience in the use of animals for scientific purposes relevant to the institution and the business of the AEC. This shall include possession of a higher degree in research or equivalent experience. If the business of the AEC relates to the use of animals for teaching only, therefore, the teacher with substantial and recent experience may be appointed.

c) Category C – A person who demonstrates commitment to, and established experience in, furthering the welfare of animals, not employed by or otherwise associated with the institution, and

not currently involved in the care and use of animals for scientific purposes. Veterinarians with specific animal welfare interest and experience may meet the requirements of this category. In the cases where a veterinarian acts as category C member, there shall be an additional category A veterinarian (i.e. one veterinarian cannot act as both categories A and C members). The person should be selected on the basis of active membership of, and endorsement by an animal welfare organization. This member should bring an animal welfare perspective to the AEC deliberations. While all members of the AECs shall consider the welfare of the animals, the category C member brings to the committee a special awareness of current community and broader animal welfare concerns.

d) Category D – An independent person(s) who does not currently and has not previously conducted scientific studies or teaching activities using animals, either in their employment or beyond their undergraduate education, and who is not an employee of the institution, except under defined circumstances (for example, tenured academic staff from non-animal scientific departments). If such an employee is appointed, the individual shall be in a senior position, and shall not be supervised by other committee members or by anyone involved in the 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 departments not including life sciences, biological sciences and health sciences 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 member should not be made internally, therefore, secretaries or administrative staff is 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 national 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.

5.3.3.2.1.3 Additional members to assist the AEC to function effectively

5.3.3.2.1.3.1 The institutions should appoint to the AEC person(s) responsible for the routine care of animals within the institution, ensuring that the people have up-to-date information of all of the various facilities.

5.3.3.2.1.3.2 The institutions may appoint additional members with necessary skills and background of value to the AEC.

5.3.3.2.2 Access to expertise

The AEC may invite people with specific expertise to provide advice, as required.

5.3.3.2.3 Balance of membership

Categories C and D members shall, together, represent at least one-third of the AEC membership. In the cases where category D members are associated with the institution, there shall be at least one category D member who is not associated with the institution.

5.3.3.2.4 Appointment, reappointment and retirement of members

5.3.3.2.4.1 The institutions shall develop procedures for the appointment, reappointment and retirement of the AEC members.

5.3.3.2.4.2 Procedures shall include the declaration of interests by prospective members and the management of conflicts of interest in making appointments.

5.3.3.2.4.3 Before appointment, all members of the AEC shall acknowledge in writing their acceptance of the terms of reference of the AEC and any requirements for confidentiality as required by the institution (see 5.2.3.1.2(d) and 5.3.3.5.3).

5.3.3.2.4.4 The institutions should ensure that the AEC members undergo appropriate induction, and have access to appropriate continuing education programmes and resources available nationally or internationally.

5.3.3.2.5 Responsibilities of the chairperson

The chairperson is responsible for impartially guiding the operation of the AEC, resolving conflicts of interest related to the business of the AEC, and representing the AEC in any negotiations with the institution's management. The chairperson should form part of the review board as referred to in 5.3.3.6.

5.3.3.2.6 Responsibilities of members

5.3.3.2.6.1 Each member is responsible for deciding whether, in their own judgement, an application or other matter under consideration by the AEC is ethically acceptable (see 4.2.3), and meets the requirements of this standard.

5.3.3.2.6.2 To fulfil this responsibility, members should

a) be familiar with this standard and other policies and guidelines relevant to the business of the AEC, and

b) provide opinions on the ethical acceptability of applications and other matters under consideration by the AEC.

5.3.3.2.6.3 During their appointment to the AEC, and before any deliberations of the AEC, members shall declare any interest that could influence the objectivity of their decision making.

5.3.3.2.6.4 Members shall maintain confidentiality regarding the content of applications and the deliberations of the AEC, in accordance with the institutional requirements.

5.3.3.2.6.5 Members shall actively partake on all deliberations of the committee discussions and attend meetings regularly (as indicated by the institutional policies). In cases where attendance is not possible, written comments on protocol reviews shall be provided to the committee.

5.3.3.3 Ensure the AEC has terms of reference

The institution shall ensure that the AEC has terms of reference that are publicly available and include the following provisions:

a) the scope of its responsibilities for ethical review, approval and monitoring of animal care and use (see 5.4);

b) its institutional accountability;

c) its mechanisms of reporting;

d) the way in which it meets the requirements for categories of minimum membership; and

e) conflict management of both the committee members and the researchers.

5.3.3.4 Provide the AEC with adequate resources

The institution shall provide the AEC with the resources required to carry out its responsibilities (see 5.4) and to maintain the AEC, and respond effectively to recommendations from the AEC regarding resources and workloads. Resources should include

a) staffing and administrative assistance, and financial resources,

b) orientation and education of the AEC members,

c) where appropriate, the reimbursement of out-of-pocket expenses or payment of an allowance to the AEC members (or both),

d) ensuring, through the AEC, that all scientific studies and teaching activities which involve the use of animals comply with the relevant national legislation (see foreword) on the use of animals for scientific studies and teaching activities, including, in particular, compliance with the relevant national legislation (see foreword) personnel authorizations for veterinary or para-veterinary procedures, and

e) provision of each AEC with facilities, powers and resources to fulfil its terms of reference and operation as set out in 5.4.

5.3.3.5 Establish procedures for the AEC governance and operation

5.3.3.5.1 General

The institutions shall establish procedures for the effective governance and operation of the AEC that enable the AEC to comply with this standard and the relevant institutional policies, and promote competent and timely ethical review of animal care and use. These procedures should include declaration of interests and management of conflicts of interest, confidentiality, appointment of and delegation of functions to the AEC executive, administrative processes, meeting procedures, communication, complaints and non-compliance, records and documentation.

5.3.3.5.2 Declaration of interests and management of conflicts of interest

Procedures for declaration of interests and management of perceived or actual conflicts of interest involving the AEC members, and experts whose advice is sought by the AEC, shall require people with a conflict of interest to remove themselves from the AEC's decision making on matters that relate to the conflict of interest.

5.3.3.5.3 Confidentiality

The institutions should develop policies for maintaining confidentiality regarding the content of applications and all deliberations of the AEC, including how members may seek advice without breaching confidentiality. This is important to allow the committee members to speak freely during meetings.

5.3.3.5.4 The AEC executive

5.3.3.5.4.1 If established, the AEC executive

a) Shall include the chairperson, a category A member and at least one member from either category C or D (see 5.3.3.2.1.2).

b) May be delegated to approve minor amendments to approved projects or activities, for ratification at the next AEC meeting. The AEC should provide guidance on the type of activity that would be a minor amendment. A minor amendment may include a change to an approved project or activity where the proposed change is not likely to cause harm to the animals, including pain, suffering or

distress. The committee may decide whether a full protocol needs to be submitted instead of it being a minor amendment.

c) May establish procedures for expedited review. The nature of research that may be expedited should be described in the procedures.

5.3.3.5.4.2 Expedited review should apply, in principle, only to research that poses no more than minimal risk of harm.

5.3.3.5.4.3 Any decision made by the AEC executive shall be reviewed by the AEC at the next quorate meeting.

5.3.3.5.5 Administrative processes

The institutions should develop policies and procedures for the submission, receipt and processing of applications and reports to the AEC, and make these policies and procedures readily available.

5.3.3.5.6 Meeting procedures

5.3.3.5.6.1 At least one member from each of the membership categories A, B, C and D shall be present throughout the meetings to establish a quorum for the conduct of a meeting. Categories C and D members, together, shall represent at least one-third of those members present.

5.3.3.5.6.2 Documented meeting procedures should include

a) timely distribution of meeting documents to the AEC members to enable members to be fully informed,

b) the conduct of quorate AEC meetings, including circumstances where a face-to-face meeting is not possible, for example, through the use of video conferencing and web conferencing or, in special circumstances, tele-conferencing,

c) management of any perceived or actual conflicts of interest that may arise (see 5.3.3.5.2),

d) frequency of meetings, which should be sufficient to allow for effective functioning of the AEC, and

e) review and approval of the new and on-going activities (see 5.4.3.2.1 to 5.4.3.2.14 (inclusive)).

5.3.3.5.7 Communication

5.3.3.5.7.1 The AEC shall clearly communicate its decisions, the reasons for its decisions and any conditions attached for an approval to the investigators in writing as promptly as possible.

5.3.3.5.7.2 The AEC should consider face-to-face meetings with the applicants to resolve issues.

5.3.3.5.8 Complaints and non-compliance

The institutions shall have procedures for dealing with complaints and non-compliance with this standard, complaints related to the AEC process, non-compliance with AEC authorized protocols and irreconcilable differences between the AEC and the investigator (see clause 8). A whistle blower policy is also needed to protect whistle blowers.

5.3.3.5.9 Records

5.3.3.5.9.1 The institutions shall ensure that records related to the AEC business are maintained, including

a) a register of all applications to the AEC, including the outcomes of deliberations,

b) minutes that record decisions and other aspects of the AEC's operation,

c) records of inspections conducted by the AEC (see 5.4.3.3.6),

d) the outcomes of the investigations into non-compliance, and

e) the reports of the annual review board findings (see 5.2.3).

5.3.3.5.9.2 Where appropriate, the institution, in consultation with the AEC, should ensure that the animal caretakers have access to records of approved projects and activities.

5.3.3.5.10 Documentation

The institutions, in consultation with the AEC, shall develop documentation for

a) application for the AEC approval to commence a project or activity in compliance with the governing principles of this standard (see 5.8),

b) follow-up review of an approved project or activity at scheduled times and when circumstances trigger additional follow-up review or inspections, including:

1) proposed amendment to an approved project or activity (see 5.8.3.4),

2) review of annual progress of an on-going project or activity,

3) unscheduled adverse events, and

4) the potential non-compliance with this standard for approved study protocols

c) reporting on an approved project or activity that has been completed or discontinued.

5.3.3.5.11 Standard operating procedures (SOPs)

5.3.3.5.11.1 The institutions, in consultation with the AEC, may allow the AEC to consider and approve standard operating procedures (SOPs) relating to the care and use of animals, including scientific procedures. Reference to SOPs can help people prepare applications to the AEC, but may make it more difficult for the AEC to apply rigour when evaluating procedures described in applications. The SOP shall only be referenced in an application under the following conditions:

a) the SOP shall have current approval from the AEC;

b) the investigators named in the application shall be trained and confirmed competent to implement the SOP; and

c) any variation to the SOP shall be described in the application and should be considered as a prompt for review of the SOP.

5.3.3.5.11.2 New SOPs shall not be used until approved by the AEC, and may be included with an application for consideration by the AEC.

5.3.3.5.11.3 If the approved SOP is not reviewed by the AEC within three years of its previous approval, approval for the SOP lapses, and the SOP cannot be used.

5.3.3.5.11.4 Approved SOPs shall be made available to all the relevant people, including the AEC members study participants and the animal caretakers.

5.3.3.6 Conduct an annual review of the operation of the AEC

5.3.3.6.1 The institution shall conduct an annual review of the operation of the AEC to ensure that it is effective and consistent with this standard and institutional policies. This shall include an assessment of the AEC's annual report (see 5.2.3.7.1 and 5.4.3.8.1 to 5.4.3.8.2 (inclusive)) and a meeting with the AEC chairperson.

5.3.3.6.2 The review board should consist of the AEC chairperson, another senior member of the institution (for example, director research) and one external reviewer as a minimum composition.

5.3.3.6.3 The reviewer should consider the following topics:

a) the exact number of animals used per species;

b) the number of animals bred per species;

c) the severity category of the studies;

d) reporting on the numbers of the type or model of research, for example, toxicology, safety;

e) the number of studies that were submitted, approved and rejected;

f) the composition of the AEC in terms of the categories;

g) the number of inspections;

h) the reason certain studies were stopped;

i) the number of reported deviations or violations and the outcome of these and the sanctions imposed;

j) the number of threatened and endangered species worked with;

k) the certification of the animals as specified by the relevant national departments (see foreword);

l) the confirmation of personnel that are certified or authorized by the relevant national council (see foreword); and

m)the numbers of genetically modified animals produced.

5.4 Responsibilities of the AEC

5.4.1 General

This clause describes the responsibilities of the AECs regarding ethical review, approval and monitoring of animal care and use in accordance with this standard. The responsibilities of institutions regarding the governance of the AEC are described in 5.3. The responsibilities of institutions regarding the care and use of animals for scientific purposes are described in 5.2.

5.4.2 Governing principles

Each person involved in the care and use of animals for scientific purposes shall consider the governing principles in clause 4 when applying this standard to their specific circumstance, in particular:

a) the institutions, the AECs, and the people involved in any aspect of the care and use of animals for scientific purposes shall be aware of and accept their responsibilities, and act in accordance with this standard (see 4.7.4.1),

b) all activities, including projects that involve the care and use of animals for scientific purposes shall

1) be subject to ethical review, approval and monitoring by the AEC,

2) commence only after approval has been granted by the AEC,

3) be conducted in accordance with the AEC approval, and

4) cease if approval from the AEC elapses, is suspended or withdrawn or period of said approval ends (see 4.7.4.2).

c) the AEC shall be satisfied that there is sufficient evidence to support a case that the proposed use of animals is justified (see 4.2.2).

5.4.3 Responsibilities

5.4.3.1 General

5.4.3.1.1 The primary responsibility of the AEC should ensure, on behalf of the institution for which it acts, that all activities relating to the care and use of animals are conducted in compliance with this standard.

5.4.3.1.2 The AEC shall

a) review applications for projects and approve only those applications that are ethically acceptable (see 4.2.3) and conform to the requirements of this standard,

b) review applications for activities associated with the care and management of animals in facilities, including procedures applicable to breeding programmes integral to the maintenance of an animal line, and approve only those activities that are ethically acceptable and conform to the requirements of this standard,

c) conduct follow-up review of approved projects and activities (see 5.3.3.5.10(b)), and allow the continuation of approval for only those projects and activities that are ethically acceptable and conform to the requirements of this standard,

d) monitor the care and use of animals, including housing conditions, practices and procedures involved in the care of animals in facilities,

e) take appropriate actions regarding unscheduled adverse events,

f) take appropriate actions regarding non-compliance,

g) approve guidelines for the care and use of animals on behalf of the institution,

h) provide advice and recommendations to the institution,

i) report on its operations to the institution,

j) ensure that adequate consideration has been given to biosecurity, biosafety and workplace safety (in other words, either directly review this information or relies on the information from another expert committee), and

k) ensure registration with the relevant national legislation (see foreword).

5.4.3.2 Review and approve new and on-going activities

5.4.3.2.1 The AEC shall provide competent, fair, consistent and timely review of applications and reports related to the care and use of animals.

5.4.3.2.2 The AEC shall make a judgement on whether the proposed use or continued use of animals is ethically acceptable. This judgement shall

a) be based on information provided by the applicant (see 5.8) that demonstrates the application of the principles outlined in clause 4, and

b) balance whether the potential effects on the well-being of the animals involved is justified by the potential benefits.

5.4.3.2.3 The AEC may approve only those projects and activities that are ethically acceptable and conform to the requirements of this standard.

5.4.3.2.4 The AEC shall consider and approve applications for new projects and activities, and consider the on-going approval for existing projects and activities, only at quorate meetings of the AEC (see 5.3.3.5.6.1 and 5.4.3.2.10).

5.4.3.2.5 Procedures should describe how applications and reports will be assessed in a manner that is fair to applicants and acceptable to all the AEC members, including the need to provide members with information in a timely manner.

5.4.3.2.6 The AEC shall base its decisions on the information it receives from the applicant in the documentation and in any direct discussions with the applicant, and may use information in addition to that obtained from the applicant.

5.4.3.2.7 The AEC may decide that

a) an application to commence a project or activity, or amend an approved project or activity, is approved with or without conditions, deferred subject to modification, or rejected,

b) following review of the annual report for an approved project or activity and possible consultation with the applicant, the approval for the project or activity is continued, suspended, modified or discontinued, and

c) an approval is suspended or withdrawn or period of said approval ends.

5.4.3.2.8 Decisions should be based on a thorough, fair and inclusive process of discussion and deliberation by the AEC members, and should be made only by those present throughout the discussion.

5.4.3.2.9 Decisions should be made on the basis of consensus. Where consensus cannot be reached after reasonable effort to resolve the differences, the AEC should explore with the applicant(s) ways of modifying the project or activity that may lead to consensus. If consensus is still not achieved, the AEC should only proceed to a majority decision after members have been allowed a period of time to review their positions, followed by further discussion.

NOTE There is one vote per category (i.e. categories A, B, C and D) and there may be no conflict of interest in any of the categories voting. In the case of a split vote within a category, the majority vote within the category becomes the category's vote; if there is a tie within a category, the category's vote is zero. The chairperson does not usually have a vote (see 5.3.3.2.1.1.1) but in cases of a tie across categories, the chairperson has a deciding vote. The additional members' (see 5.3.3.2.1.3) input is crucial, to share specialized information whereby the category members can make informed decisions.

5.4.3.2.10 For decision making, members with a conflict of interest shall withdraw from the meeting. Once such members have withdrawn, the remaining members shall constitute a quorum as defined

in 5.3.3.5.6.1, i.e., one member from each of the membership categories A, B, C and D, with categories C and D together representing at least one-third of members present.

5.4.3.2.11 Decisions of the AEC shall be made as promptly as possible.

5.4.3.2.12 Pilot studies, where proposed, should be regarded as integral to the overall project, especially to enable assessment of the feasibility of the study and the potential for refinement and reduction. The AEC shall assess the pilot study results and they shall determine the criteria for further application approval.

5.4.3.2.13 When considering approval for the reuse of animals, the AEC shall take into account

a) the pain, suffering, distress and lasting harm, and any potential long-term or cumulative effects, caused by previous activities and conditions, and shall consider the lifetime experience of the animal,

b) the time allowed for recovery of the animals between activities,

c) whether an animal has fully recovered from the previous activities,

d) the pain, suffering, distress and lasting harm likely to be caused by the next and subsequent activities, and

e) the total time over which an animal will be used.

5.4.3.2.14 In determining the duration of approval for individual projects, the AECs should take into account the number of years for which the project is funded, any milestones or stages outlined in the project, and any formal agreements between the institution and funding bodies.

5.4.3.3 Monitor the care and use of animals

5.4.3.3.1 The AEC (or the appointed AEC members) monitors the care and use of animals by physically inspecting animals, animal housing and the conduct of procedures, as well as by reviewing records and reports.

5.4.3.3.2 The AEC shall monitor all activities relating to the care and use of animals (including the acquisition, transport, breeding, housing and husbandry of animals) on a regular and on-going basis to assess compliance with this standard and decisions of the AEC. The AEC shall ensure that identified problems and issues receive appropriate follow-up and, if necessary, report suspected breaches of the standard to the institution.

5.4.3.3.3 The AEC shall monitor activities that are likely to cause pain, suffering, distress or lasting harm at an early phase during the conduct of the activity. This requirement should be a condition of approval for the project or activity. These activities could include, but are not limited to, the study of pain, responses to stressors, models of human and animal diseases, or attempts to change behaviour by physical or chemical means.

5.4.3.3.4 All categories of the AEC should participate in research animal facility inspections or site inspections.

5.4.3.3.5 The AEC should determine the frequency and timing of inspections. Influencing factors include the number and accessibility of sites, the number and types of projects and activities, and whether inspections can be combined with scheduled AEC meetings. In addition, the AEC may decide that certain projects or activities require more frequent inspection than others, guided by, for example, the severity grade and historic audit results. Inspections may be announced or unannounced.

5.4.3.3.6 The AEC shall maintain records of inspections that include the names of attendees, observations, any identified problems, recommended actions, on-going or outstanding issues, and outcomes (see 5.3.3.5.9.1(c)).

5.4.3.3.7 The AEC procedures should cover the delegation of authority to suitably qualified people to monitor animal care and use, including projects and activities conducted at remote sites (for example, fieldwork). Procedures should include how reports of such monitoring should be provided to the AEC (for example, using still or video images).

5.4.3.4 Take action regarding unscheduled adverse events

The AEC shall take appropriate action in response to unscheduled adverse events to ensure that animal well-being is not compromised, the issue is addressed promptly, and activities that have the potential to adversely affect animal well-being cease immediately (see 5.2.3.3(e)(4)). Actions may include consulting with the relevant people and, where necessary, suspending or withdrawing approval for the project or activity.

5.4.3.5 Take action regarding non-compliance

When projects or activities that are in breach of the standard are detected, the AEC shall ensure that:

a) Actions are taken to ensure that animal well-being is not compromised, the issue is addressed promptly, and activities that have the potential to adversely affect animal well-being cease immediately (see 8.3.1.1(a) and 8.3.1.2(a)). Actions may include suspending or withdrawing approval for the project or activity.

b) Actions are taken to address the issues in consultation with the person(s) involved.

c) When considered necessary, such matters are referred to the institution for action.

d) Non-compliance receives appropriate follow-up.

5.4.3.6 Approve guidelines for the care and use of animals

The AEC shall consider approval of guidelines for the care and use of animals that are referred to it by the institution (see 5.2.3.3(e)).

5.4.3.7 Provide advice and recommendations to the institution

The AEC shall provide advice and recommendations to the institution regarding the care and use of animals for scientific purposes conducted on behalf of the institution, and strategies required to ensure that the requirements of this standard are maintained and that matters affecting animal well-being are addressed.

5.4.3.8 Report to the institution

5.4.3.8.1 The AEC shall submit a written report on its operations, at least annually, to the governing body of the institution(s) for which it acts.

5.4.3.8.2 The report should advise on

a) the numbers and types of projects and activities assessed and approved or rejected,

b) the physical facilities for the care and use of animals by the institution,

c) actions that have supported the educational and training needs of the AEC members and people involved in the care and use of animals,

d) administrative, funding or other difficulties experienced, and

e) any matters that may affect the institution's ability to maintain compliance with this standard and, if appropriate, suitable recommendations.

5.5 Responsibilities of the investigators

5.5.1 General

This clause relates to the responsibilities of the investigators, i.e. the researchers, the teachers, the undergraduate and postgraduate students involved in research projects, and the people involved in product testing, environmental testing, production of biological products and wildlife surveys.

5.5.2 Governing principles

Each person involved in the care and use of animals for scientific purposes shall consider the governing principles in clause 4 when applying this standard to their specific circumstance, in particular

a) respect for animals shall underpin all decisions and actions involving the care and use of animals for scientific purposes (see 4.2.1),

b) the obligation to respect animals, and the responsibilities associated with this obligation, apply throughout the animal's lifetime, including acquisition, transport, breeding, housing, husbandry, use of the animal in a project, and provisions for the animal at the conclusion of their use (see 4.2.4),

c) the institutions, the AECs and the people involved in any aspect of the care and use of animals for scientific purposes shall be aware of and accept their responsibilities, and act in accordance with this standard (see 4.7.4.1),

d) all activities, including projects that involve the care and use of animals for scientific purposes shall

   1) be subject to ethical review, approval and monitoring by the AEC,

   2) commence only after approval has been granted by the AEC,

   3) be conducted in accordance with the AEC approval,

   4) cease if approval from the AEC is suspended or withdrawn or period of said approval ends (see 4.7.4.2).

5.5.3 Responsibilities

5.5.3.1 General

5.5.3.1.1 The investigators shall have personal responsibility for all matters that relate to the well-being of animals that they use, including their housing, husbandry and care. This responsibility extends from the moment the animal is issued for the study, throughout the period of use, as approved by the AEC, until provisions are made for the animal at the conclusion of their use.

5.5.3.1.2 The investigators shall comply with all the relevant national legislation (see foreword).

5.5.3.1.3 The investigators shall only consider using animals when they are satisfied that a case can be made that the proposed use is ethically acceptable, based on whether such use demonstrates the

principles in 4.2.1, and balancing whether the potential effects on the well-being of the animals involved is justified by the potential benefits (see 4.2.3).

5.5.3.1.4 The investigators should seek advice and information from the relevant experts, including other experienced scientists, veterinarians, animal caretakers statisticians or specialists in laboratory animals, livestock or wildlife, when necessary.

5.5.3.1.5 The investigators shall

a) apply the principles of this standard (see clause 4) in all aspects of the care and use of animals, including planning, conducting and reviewing projects,

b) follow relevant policies and procedures established by the institution and the AEC (see 5.2.3.3(d) and 5.2.3.3(e)),

c) apply for and obtain written approval from the AEC before commencing a project that involves the use of animals, or an amendment to an approved project, include where relevant consent form,

d) conduct a project involving the use of animals in accordance with the conditions and requirements of the AEC approval, and cease the project if approval from the AEC is suspended or withdrawn or period of said approval ends,

e) undertake education and training, including continuing education and competency assessment, in accordance with the institutional and the AEC policies and procedures,

f) ensure that procedures using animals are performed competently,

g) maintain records of the care and use of animals,

h) ensure that the animals are monitored at least once daily, including weekends and public holidays

i) report to the AEC as required, and

j) declare, through an appropriate mechanism (for example, biosafety committee), all potential hazards from the research project.

5.5.3.1.6 A person who has the ultimate responsibility for the care and use of animals in a project shall be identified. This person shall

a) ensure that all people involved in the project understand and accept their roles and responsibilities,

b) ensure that procedures and resources are in place so that all people involved in the care and use of animals in the project can meet their responsibilities, including their education, training and supervision, as appropriate, and

c) be competent and experienced with respect to the well-being of animals used in the project.

NOTE This person does not relieve the individual responsibility of each investigator working with animals in the project.

5.5.3.1.7 For projects involving xenotransplantation, the investigators shall ensure that measures are in place to minimize the potential for xenosis, including the appropriate screening of source animals, management of bio-hazardous waste and emergency plans for the management of adverse outcomes. The investigators should consider collecting and retaining tissue samples from the source and recipient animals.

5.5.3.1.8 The researchers should ensure that the publication of animal research studies, provides sufficient information to allow replication of the work and confidence in the results, including details of the animals, husbandry, study design, sample sizes, statistical analysis, and experimental methods.

5.5.3.2 Planning projects

5.5.3.2.1 When planning projects, the investigators shall only consider using animals when

a) the use of animals is justified (see 4.2.1(a) and 4.3.1 to 4.3.2 (inclusive)),

b) high standards of scientific integrity are applied (see 4.2.1(d) and 4.6.1 to 4.6.3 (inclusive)),

c) the four Rs are applied at all stages of the project (see 4.2.1(e) and 4.7.1.1 to 4.7.3.6 (inclusive)),

d) measures are taken to ensure that the animals' environment and management are appropriate for the species and support the animals' well-being (see 4.2.1(b) and 4.4.1 to 4.4.2 (inclusive))

e) the project is designed to avoid or minimize harm, including pain, suffering, distress and lasting harm, to the animals (see 4.2.1 (c) and 4.5.1 to 4.5.4 (inclusive)), and

f) all people involved in the care and use of animals in the project understand and accept their roles and responsibilities (see 4.2.1(e) and 4.7.4.1 to 4.7.4.2 (inclusive)).

5.5.3.2.2 When planning projects, the person with the ultimate responsibility to conduct the project shall be identified (see 5.5.3.1.6).

5.5.3.2.3 During planning, the investigators shall consider the following factors and be satisfied that

a) The project has scientific or educational merit.

b) The aims of the project cannot be achieved entirely or in part without the use of animals.

c) The potential benefits justify the potential effects on the well-being of the animals involved.

d) Particular justification is provided for activities that involve severe compromise to animal well-being and for which the four Rs cannot be fully applied for the activity to proceed, and for activities that involve the use of non-human primates (see 5.8.3.2(e)).

NOTE Use animals only when justified (see 4.2.1(a) and 4.3.1 to 4.3.2 (inclusive)).

e) The choice of species, source of the animals and biological status of the animals (for example, genetic, nutritional, microbiological and general health status) are suited to the purpose of the project.

f) Factors that may contribute to variability of results are taken into account, including the biological status of the animals and their living conditions (for example, physical, environmental and social conditions).

g) Unintended adverse impacts on animal well-being that may confound experimental data are avoided or minimized.

h) The methods and procedures to be used in accordance with current best practice and are appropriate for the purpose of the project.

NOTE Apply high standards of scientific integrity (see 4.2.1(d) and 4.6.1 to 4.6.3 inclusive)).

i) Steps are taken to consider and apply the four Rs at all stages of the project.

j) The project is designed to use the minimum number of animals to obtain valid data or achieve educational objectives, and to satisfy good statistical design.

NOTE Apply the four Rs (see 4.2.1(e) and 4.7.1.1 to 4.7.3.6 (inclusive)).

k) Living conditions and management that are appropriate for the species are available, including suitable housing facilities.

l) Any special requirements for the care and management of the animals are met (see clause 10).

m) Details and justification are provided for care and management of the animals that does not accord with current best practice (see 4.4.2).

n) Procedures are in place for monitoring and managing animal health during the project (see 10.6.3.1).

NOTE Support the well-being of animals (see 4.2.1 (b) and 4.4.1 to 4.4.2 (inclusive)).

o) Known and potential causes of adverse impact on the well-being of animals are identified, and strategies to avoid or minimize harm, including pain, suffering, distress and lasting harm, are developed (see 6.3). Experimental and non-experimental factors shall be considered.

p) A pilot study is incorporated into the design of the project if the potential impact on the animal cannot be predicted on the basis of available evidence, to allow staged assessment of the impact on animal well-being and the development of strategies to avoid or minimize any adverse impact.

q) The well-being of the animals is monitored and assessed by competent people at least once daily including weekends and public holiday.

NOTE Avoid or minimize harm, including pain, suffering, distress and lasting harm (see 4.2.1(c) and 4.5.1 to 4.5.4 (inclusive)).

r) All people involved in the proposed project understand and accept their roles and responsibilities in the project and the relationship of their roles and responsibilities to those of other people involved in the project.

s) Procedures are performed competently, by people competent for the procedures or under the direct supervision of a person competent to perform the procedures (in accordance with the provisions of the relevant national legislation (see foreword) relating to personnel authorizations for veterinary or para-veterinary procedures), and provisions are made for the education, training and supervision of people nominated on the application, as appropriate.

t) The conduct of the proposed project is feasible, after consultation with the facility manager if appropriate, and taking into consideration the available resources (for example, funding, personnel, physical, equipment), the type and availability of animals required, and requirements to support the well-being of the animals. Funding for pilot studies, where required by the AEC, should be included in financial budgets as an integral part of the scientific design.

u) Appropriate approvals, and any administrative requirements of the institution and the AEC, are in place. These could include permits and licences, documentation to certify the biological status of animals, biosafety, work health and safety considerations, and arrangements for projects conducted at more than one institution.

NOTE Accept responsibilities (see 4.2.1(d) and 4.7.4.1 to 4.7.4.2 (inclusive)).

5.5.3.2.4 The investigators shall notify their institutional AEC in writing if they are involved in collaborative studies using animals at another institution, or if they are named in an application to the AEC of another institution (see 5.7.4.2). It remains the prerogative of the institution to decide if these project(s) require further approval from their institution AEC or if inter-institution agreements are to be signed.

5.5.3.3 Obtaining approval from an animal ethics committee

5.5.3.3.1 Before commencing a project, or an amendment to an approved project, the investigators shall

a) submit an application to the AEC, and

b) obtain written approval from the AEC.

5.5.3.3.2 The investigators shall follow institutional and AEC policies and procedures when submitting an application to the AEC (see 5.3.3.5.5 and 5.8), and provide information in the application as outlined in 5.8.3.2 and 5.8.3.4.

5.5.3.3.3 The investigators shall use plain English in the application to the AEC to ensure that all AEC members are provided with sufficient information to participate effectively in the assessment of the application.

5.5.3.4 Conducting and reviewing projects

5.5.3.4.1.1 Clause 6 shall be read in conjunction with 5.5.3.4.1.2 to 5.5.3.4.5 (inclusive).

5.5.3.4.1.2 The investigators shall conduct all aspects of a project in accordance with the conditions and requirements of the AEC approval and any subsequent amendments approved by the AEC.

5.5.3.4.1.3 The investigators shall cease the conduct of a project or any part of a project if approval from the AEC is suspended or withdrawn or period of said approval ends.

5.5.3.4.1.4 The investigators shall apply high standards of scientific integrity by

a) confirming that animals are suitable for their proposed use at the time they are supplied or procured for that use, and

b) ensuring that procedures involving animals are in accordance with the current best practice (see 6.4).

5.5.3.4.1.5 The investigators shall consider and support the well-being of animals used in the project in terms of the cumulative effects of the animal's lifetime experience. At all stages during the project, the investigator shall ensure that the animal's environment and management are appropriate for the species and support the animal's well-being.

5.5.3.4.1.6 The investigators shall ensure that animal care is provided by an adequate number of competent people (see 5.6.3.1.1(b)).

5.5.3.4.1.7 The investigators shall take steps at all times to safeguard the well-being of animals by avoiding or minimizing known or potential causes of pain, suffering, distress and lasting harm to the animals. These steps include

a) Using methods that cause the least pain, suffering, distress and lasting harm.

b) Ensuring that procedures are performed competently, and that the investigators are

1) competent for the procedures they perform, or

2) under the direct supervision of a person who is competent to perform the procedures (see 5.5.3.2.3(s))

c) Implementing and reviewing strategies to detect, avoid and minimize any pain, suffering, distress and lasting harm in the animals (see 6.3).

d) Ensuring that animals used are identified either individually or in groups (see 6.4.4) ensuring that people involved in the care and use of animals in the project are knowledgeable about the normal behaviour and signs of pain, suffering, distress and lasting harm for the species they will use.

e) Ensuring that animals are monitored and assessed at all stages of the project for signs of pain, suffering, distress and lasting harm, including deviations from normal behaviour (see 6.3.3.3.1 to 6.3.3.3.2 (inclusive)). Such monitoring and assessment shall be conducted at a frequency sufficient to detect such signs at an early stage, as determined by the procedure, but at least once per day, to ensure that the planned endpoints are detected early, as required by the AEC.

f) Maintaining records of monitoring and assessment of animal well-being (see 5.5.3.5.1 to 5.5.3.5.3 (inclusive)) and 6.3.3.3.3).

g) Taking prompt action based on the monitoring and assessment of animal well-being, in accordance with the intervention points and humane endpoints approved by the AEC (see 6.3.3.3.4).

h) Taking prompt action, including alleviating pain, suffering, distress and lasting harm and promptly notifying the AEC, in response to unscheduled adverse events and emergencies, in accordance with institutional and AEC policies and procedures (see 5.2.3.3(e)(4) and 6.3.3.3.5 to 6.3.3.3.6 (inclusive)). Alleviating unanticipated pain, suffering, distress and lasting harm shall take precedence over an individual animal reaching the planned endpoint of the project, or the continuation or completion of the project. If necessary, animals shall be humanely killed without delay.

i) Ensuring the appropriate use of pharmacological and non-pharmacological means to minimize pain, suffering, distress and lasting harm (see 6.4.6.1 to 6.4.6.8 (inclusive)). Use of pharmacological agents such as anaesthetics, analgesics and sedatives shall be appropriate to the species, the individual animal (for example, age, physiological status) and the scientific aims, and shall be consistent with current veterinary or medical practice. Anaesthesia and analgesia shall be used for procedures that are likely to cause pain of a kind and degree for which anaesthesia and analgesia would normally be used in veterinary or medical practice.

5.5.3.4.1.7 The investigators shall continually consider how to apply the four Rs during the conduct of the project (see 4.7.1.1 to 4.7.3.6 (inclusive)). Any subsequent amendments to the approved project shall only proceed following approval from the AEC.

5.5.3.4.1.8 The Investigators shall accept responsibilities and

a) Act in accordance with their role and responsibilities in the project.

b) Ensure that the scope of monitoring the well-being of the animals at all stages of their care and use in the project is clearly outlined and communicated to all parties. Depending on the type of project, this may include monitoring by the animal caretakers.

5.5.3.4.2 Provisions for animals at the conclusion of their use and disposal of carcasses and waste material

5.5.3.4.2.1 The investigators shall take prompt action regarding provisions for animals at the conclusion of their use, in accordance with procedures and protocols approved by the AEC (see 6.4.20.1 to 6.4.20.6 (inclusive)).

5.5.3.4.2.2 The investigators shall use humane procedures for killing an animal that are appropriate to the species and circumstances (see 6.4.21.1 to 6.4.21.2 (inclusive)).

5.5.3.4.2.3 Unless otherwise required (for example, as part of a project or for the investigation of a disease outbreak), the investigators shall ensure that all carcasses and tissues from animals that have died or been humanely killed are disposed of in a sanitary and appropriate manner (see 5.2.3.1.2(g)).

5.5.3.4.2.4 The investigators should ensure that, if practicable, tissue samples from animals that have died or been humanely killed are provided or made available to other investigators for their work, or deposited in a tissue bank for subsequent distribution.

5.5.3.4.3 Projects involving hazards

For projects that involve hazards to other animals and humans, the investigators shall ensure that

a) all personnel is aware of these hazards, and any potential pathogenic effects from these hazards, and

b) appropriate procedures are implemented for quarantining and handling animals that pose a risk to other animals and to humans because of naturally acquired or experimentally induced infectious disease.

5.5.3.4.4 Creation and breeding of new animal lines where the impact on animal well-being is unknown or uncertain

5.5.3.4.4.1 The creation and breeding of a new animal line, including genetically modified and cloned animals, where the impact of the genotype on animal well-being is unknown or uncertain is regarded as a scientific purpose. Persons responsible for animals involved in such projects are regarded as the investigators. Their responsibilities extend until the impact on animal well-being is known and the AEC has approved the final report on the generation of a new animal line. After this the AEC approval, the new line can be treated as breeding stock (see 5.6 and 6.4.11).

5.5.3.4.4.2 The investigators shall

a) Not generate a new animal line using genetic modification if a similar, suitable animal model is available to the investigator or a relevant in vitro method can be used to achieve the aims of the project.

b) Ensure that the AEC approval is in place from the start of the process until the impact of the genotype on well-being is known, and data on mortality, morbidity and population health of the new line are available. Procedures used for creating and breeding these animals shall be regarded as part of a protocol that is reviewed by the AEC.

c) Use methods to support and safeguard the well-being of the animals involved (see 6.4.11).

d) Advise the AEC when the clinical status of the animals changes to a kind or degree that was not predicted.

e) Maintain records of the number of animals used to create and maintain the new animal line, and the lineage and health status of the animals. Following approval from the AEC for the new animal line to be treated as breeding stock, the facility manager or animal caretaker is responsible for records of the maintenance of the animal line (see 5.6.3.5.1, 5.6.3.5.3 and 5.6.4.2(j)).

f) Ensure that reports are provided to the AEC (see 5.5.3.6), including:

1) regular reports on the monitoring of a new animal line at a frequency determined by the AEC, and

2) a final report on the generation of the new animal line.

g) Ensure that animals and their offspring are not sold, or transferred to another facility, unless the recipient of the animals accepts full responsibility for completion of the phenotype assessment, where applicable.

5.5.3.4.5 Using privately owned animals

For projects involving the use of privately owned animals (for example, livestock or companion animals), the investigators shall:

a) Submit a template consent form to the AEC application and should be considered by the AEC in their deliberation. The informed consent form should be in a non-technical language that is easily understandable and should address the following:

1) title of project, researcher, organization and funder,

2) owner and animal details,

3) brief description of the project,

4) potential benefits of projects, including benefits to patient,

5) dangers of the project, including side effects and potential of fatal outcomes,

6) implications if it is placebo controlled,

7) restitution in cases of injury including cost of care and restitution values (testing requirements, for example, a post-mortem for payment of claims needs to be stated),

8) compensation, including future treatment in case of project success,

9) payments made by research project and costs borne by owner,

10) in case of a multi-site study, the court which has jurisdiction has to be stated,

11) statement of ethical approval,

12) publication of results and personal details,

13) the right to withdraw the animal from the study, and

14) post-mortem examination.

b) Ensure that all people involved in the care and use of such animals are aware of and accept their responsibilities relating to the animals.

c) Ensure that people responsible for the daily management of the animals during the project are familiar with and understand the standard, and are competent.

d) Provide the owner of the animal with a document, to be included in the application to the AEC, clearly stating the details and duration of the owner's responsibilities. The owner should acknowledge their acceptance of these responsibilities in writing.

5.5.3.5 Maintaining records

5.5.3.5.1 The investigators shall maintain records of the care and use of all animals, and make such records available to the institution, the AEC and authorized expert independent reviewers.

5.5.3.5.2 The investigators shall ensure that records of welfare monitoring and assessment of all animals are in accordance with clauses 6.3.3.3.2 to 6.3.3.3.3 (inclusive), in accordance with the approved AEC protocol.

5.5.3.5.3 The investigators shall ensure that records include the following:

a) details for each individual animal;

b) the origin or source of the animals and provisions for the animals at the conclusion of their use;

c) the number of animals used;

d) details of all procedures, including dates, substances administered with dosages, analgesia and anaesthesia, any deviations from the approved AEC protocol, and any non-conformances relating to the care and use of animals and unscheduled outcomes;

e) the condition of the animal, any adverse impact on animal well-being and actions taken as a result;

f) any additional information requested by the AEC;

g) names of people performing the procedures and entering the records;

h) names and contact details of people responsible for monitoring and emergency incidents;

i) the reason, cause and time of an animal's death; and

j) post mortem results.

5.5.3.5.4 When activities involve genetically modified animals, records shall include the number of animals used for the creation and maintenance of genetically modified animals, and the lineage and health status of the animals.

5.5.3.6 Reporting

The investigators shall provide the following to the AEC in accordance with the AEC and the institutional policies and procedures (see 5.3.3.5.5 and 5.3.3.5.10):

a) an annual report for an approved project, regardless of the duration of the AEC approval for the project;

b) prompt notification of any unscheduled adverse events (see 5.2.3.3(e)(4));

c) final report on outcomes as soon as practicable after completion or discontinuation of a project;

d) reports on the creation and maintenance of genetically modified animals (see 5.5.3.4.4.2(d));

e) incident reports on any deviations from the approved AEC protocol, and any unscheduled outcomes and non-conformances relating to the care and use of animals; and

f) any other reports as required by the AEC.

5.6 Responsibilities of the animal caretakers

5.6.1 General

The number, training and competence of animal caretakers are important factors that contribute to high quality animal care. This sub-clause relates to the responsibilities of people involved in the care of animals that are used for scientific purposes, including during their acquisition, capture, transport, breeding, housing and husbandry.

5.6.2 Governing principles

Each person involved in the care and use of animals for scientific purposes shall consider the governing principles in clause 4 when applying this standard to their specific circumstance; in particular:

a) respect for animals shall underpin all decisions and actions involving the care and use of animals for scientific purposes (see 4.2.1).

b) the obligation to respect animals, and the responsibilities associated with this obligation, apply throughout the animal's lifetime, including acquisition, transport, breeding, housing, husbandry, use of the animal in a project, and provisions for the animal on completion of their use (see 4.2.4).

c) the institutions, the AECs and the people involved in any aspect of the care and use of animals for scientific purposes shall be aware of and accept their responsibilities, and act in accordance with the standard (see 4.7.4.1).

d) all activities and animal procedures performed by animal caretakers, including projects that involve the care and use of animals for scientific purposes shall

   1) be subject ethical review, approval and monitoring by the AEC,

   2) commence only after approval has been granted by the AEC,

   3) be conducted in accordance with the AEC approval,

   4) cease if approval from the AEC is suspended or withdrawn or period of said approval ends (see 4.7.4.2)

e) breeding of animals shall be managed to avoid or minimize the production of excess animals (see 4.7.2.7).

5.6.3 Responsibilities

5.6.3.1 General

5.6.3.1.1 The scope of responsibilities of people who provide care to animals is determined by their role and the stage of the animal use:

a) Before an animal is supplied to an approved project for which the investigator is responsible, responsibility for the well-being of the animal rests with the person who is engaged by the institution to provide care for the animals (for example, facility manager, animal technician, stock handler).

b) The investigator is responsible for the well-being of an animal throughout the period of use of the animal in the approved project, until provisions are made for the animal at the conclusion of their use (see 5.5.3.1.1). The investigator shall ensure that an adequate number of competent people can provide care for the animals (for example, animal technicians, stock handlers, investigators). If the investigator acts as an animal caretaker during this period, their responsibilities include those of an animal caretaker.

5.6.3.1.2 The animal caretakers shall, within the scope of their responsibilities

a) apply the principles of this standard in all aspects of the care of animals (see clause 4),

b) follow relevant policies and procedures established by the institution and the AEC (see 5.2.3.3(d)and 5.2.3.3(e)),

c) undertake activities in accordance with the conditions and requirements of approval from an AEC,

d) take measures to ensure that the animals' environment and management are appropriate for the species and the individual animal, and support the animals' well-being,

e) ensure that steps are taken to safeguard animal well-being by avoiding and minimizing harm, including pain, suffering, distress and lasting harm, to the animals,

f) consider applying the four Rs in all aspects of the care of animals for which they are responsible,

g) ensure that their duties are performed competently,

h) liaise with the investigators, the relevant project team members, the facility managers and the relevant animal unit personnel on all matters relevant to the well-being of the animals involved including animals found dead, or in pain, suffering or distress,

i) maintain records of the care of animals, and

j) report to the veterinarian, the facility manager or the AEC as required.

5.6.3.1.3 If more than one person is responsible for the care of animals (for example, animal technicians or other veterinary para-professionals caring for animals in one or more animal breeding and holding facility, team of animal technicians and researchers caring for animals in a project, team of researchers and wildlife caretakers involved in the care of wildlife in a research project, several teachers and students caring for animals in a school), a person shall be identified who has ultimate responsibility for the care of those animals. Depending on the situation, and as determined by the AEC or institutional policy, this person may be the facility manager, or the investigator with ultimate responsibility for a project. Identification of a person with ultimate responsibility for the care of animals does not relieve the individual responsibility of each person who provides care for animals.

5.6.3.2 Support animal well-being

Animal caretakers shall

a) ensure that animals are cared for and managed so that species specific or strain-specific physiological and behavioural needs are met,

b) use procedures and practices that are based on current best practice (see 4.4.2), and

c) ensure that the health and biosecurity status of animals is maintained in a manner that safeguards animal well-being and meets the requirements of their proposed use, in accordance with institutional and AEC policies and procedures (see 10.6.3.1).

5.6.3.3 Avoid or minimize harm, including pain, suffering, distress and lasting harm, to animals

5.6.3.3.1 Animal caretakers shall

a) Ensure that their duties are performed competently, and be

   1) competent for the duties they perform, or

   2) under the supervision of a person competent to perform those duties

b) Monitor and assess the well-being of animals for which they are responsible (see 5.6.3.1.1) with sufficient frequency to ensure that harm, including pain, suffering, distress and lasting harm, is promptly detected and managed (see 6.3.3.3.1 to 6.3.3.3.2 (inclusive)). Where animal caretakers are involved in the monitoring and assessment of animals after they have been supplied to an approved project, the investigator shall ensure that the scope and responsibilities for day-to-day monitoring are clearly outlined and communicated to all parties.

c) Maintain records of monitoring and assessment of animal well-being (see 6.3.3.3.3).

d) Take prompt actions based on the monitoring and assessment of animal well-being and in response to unscheduled adverse events and emergencies, in accordance with the institutional policies and procedures, and procedures approved by the AEC (see 5.2.3.3(e)(4) and 6.3.3.3.4 to

e) 6.3.3.3.6 (inclusive)), including liaising with the investigators and seeking veterinary advice.

5.6.5.3.2 If an emergency welfare intervention is considered necessary for an animal allocated to a project (for example, treatment or humane killing of an animal), animal caretakers shall take reasonable steps to first contact the responsible investigator. However, the welfare of the animal shall be the priority at all times and may necessitate immediate intervention. Animal caretakers shall promptly advise the responsible investigator of actions taken and the reasons for emergency interventions. Reporting of the event to the AEC, and responsibility for such reporting, shall be in accordance with the institutional and the AEC policies and procedures (see 5.2.3.3(e)(4)).

5.6.3.4 Ensure provisions for animals at the conclusion of their use, and disposal of carcasses and waste material

5.6.3.4.1 The animal caretakers shall take prompt action regarding provisions for animals at the conclusion of their use, in accordance with procedures and protocols approved by the AEC (see 6.4.20.1 to 6.4.21.2 (inclusive)).

5.6.3.4.2 The animal caretakers shall use humane procedures for killing an animal that are appropriate to the species and circumstances as approved by the AEC (see 6.4.21.1 to 6.4.21.2 (inclusive)). The animal caretakers shall

a) be properly trained and have a valid relevant qualification sufficient to ensure that they are fully competent in the methodology employed and that the resulting death meets the welfare standards set out elsewhere in this standard, and

b) use humane procedures for killing an animal that are appropriate to the species and circumstances as approved by the AEC (see 6.4.21.1 to 6.4.21.2 (inclusive)).

5.6.3.4.3 Unless otherwise required (for example, as part of a project or for the investigation of a disease outbreak), the animal caretakers shall ensure that all carcasses and tissues from animals that have died or been humanely killed are disposed of in a sanitary and appropriate manner.

5.6.3.4.4 Animal caretakers should ensure that, if practicable, tissue specimens from animals that have died or been humanely killed are provided or made available to the investigators for their work, or deposited in a tissue bank for subsequent distribution.

5.6.3.5 Maintain records

5.6.3.5.1 The animal caretakers shall maintain records of the care and monitoring of animals and, for breeding facilities, the health status and breeding performance of animals (see 6.3.3.3.3, 10.6.3.7 and 5.5.3.4.4.2(d)). The animal caretakers shall make these records available to the institution, the AEC, the authorized external reviewers and, if relevant, the investigators.

5.6.3.5.2 Records of animal monitoring shall be sufficient to enable the AEC to verify that the well-being of animals has been monitored in accordance with AEC requirements and as stipulated in the protocol, and allow review and critical investigation of the cause(s) of and responses to unscheduled adverse events as a basis for future prevention strategies.

5.6.3.5.3 The animal caretakers should ensure that records relating to health status and breeding performance include the following

a) the source, care, supply, movement between locations and use of the animals;

b) details of all diseases in the facility;

c) the fertility, fecundity, morbidity and mortality rates of breeding colonies; and

d) the health status, genetic constitution and physical environment of the animals.

5.6.4 People managing and supervising breeding and holding facilities

5.6.4.1 The person responsible for the overall management of a facility used for breeding and holding animals (the facility manager) shall be competent, with appropriate animal care or veterinary qualifications or authorization or experience. The person providing oversight of the programme of veterinary care, including the care, husbandry and health of animals and biosecurity in a facility, shall be competent and hold appropriate veterinary qualifications or authorization.

5.6.4.2 The facility manager, with support as required from the institution and other staff members, and advice from veterinarians, shall:

a) Apply for and obtain written approval from the institution's AEC for all activities associated with the care and management of animals in the facility, including procedures applicable to breeding and related programmes that are integral to the maintenance of an animal line (see also 5.5.3.4.4.1 to 5.5.3.4.4.2 (inclusive)), and for any amendments to such activities (see 5.3.3.5.5 and 5.8).

b) Ensure that activities are implemented and conducted in accordance with the conditions and requirements of the AEC approval, and cease if approval from the AEC is suspended or withdrawn or period of said approval ends.

c) Ensure that all people involved in the care of animals at the facility understand, accept and fulfil their role and responsibilities, and are competent.

d) Ensure that procedures and resources are in place so that all people involved in the care of animals can meet their responsibilities, including education, training and supervision of staff, as appropriate.

e) Ensure that quality management is promoted in the facility through the systems and procedures in place, and manage the day-to-day care of animals.

f) Arrange for experienced veterinary services in a timely manner, and ensure that staff follow veterinary advice regarding care, husbandry and health of animals, and biosecurity, in the facility.

g) Ensure the development and regular review of procedures for the care and management of animals that accord with current best practice (see 4.4.2, 6.4 and 10.2 to 10.5 (inclusive)).

h) Ensure that the well-being of animals for whom they are responsible is monitored on a day-to-day basis by a competent person, and that appropriate actions are taken in accordance with both the institutional and the AEC policies and procedures, and actions documented in animal care procedures approved by the AEC (see 6.3.3.3.1 to 6.3.3.3.6 (inclusive) and 10.6.3.1).

i) Ensure that the necessary permits or approvals relating to the holding and supply of animals are in place. These may include permits or approvals from the relevant national and provincial legislation (see foreword); and may relate to the use of wildlife or genetically modified animals, or the importation of animals.

j) Ensure regular assessment of the health status and breeding performance of all animals in accordance with current best practice, maintain appropriate records of this assessment, and make these records available to investigators, the AEC, the institution and the authorized external people (see 5.6.3.5.1 to 5.6.3.5.3 (inclusive) and 10.6.3.7).

k) Liaise between investigators and facility staff, including informing investigators of any intended changes to the holding conditions for animals that may affect their studies.

l) Ensure that animals are suitable for their proposed use, and identify suitable animals for supply to a project (see 4.6.3, 6.3.3.1.5,10.3 and 10.5.1(c).

m) Communicate with the AEC regarding the management of the facility.

n) Ensure that reports are provided to the AEC in accordance with AEC and institutional policies and procedures (see 5.3.3.5.10), including:

   1) an annual report of activities; and

   2) prompt notification of unscheduled adverse events relating to animals for which the facility manager is responsible (see 5.2.3.3(e)(4))

o) Ensure that staff are advised of the work health and safety issues associated with the animals under their care and the precautions they shall take, in accordance with the institutional procedures (see 5.2.3.3(d) and 5.2.3.6(c)(1)).

5.6.4.3 The facility manager should contribute to the development and maintenance of the institution's animal care policies and procedures, including those covering quality management.

5.6.4.4 When animals for whom they are responsible are ill or injured, or show unscheduled abnormalities, the facility manager shall

a) ensure provision of prompt diagnosis and treatment under veterinary supervision and control (see 5.2.3.3(d)),

b) ensure appropriate actions in response to any subsequent report from the veterinarian on problems that may require changes to the management or care of the animals (or both) in the facility, and

c) for animals that die unscheduled deaths, ensure that institutional and AEC policies and procedures are followed regarding the conduct of a necropsy and access to diagnostic services when samples are collected for ancillary testing (see 5.2.3.3(e)(4)).

5.7 Other responsibilities of the institutions, the investigators and the AEC

5.7.1 General

This clause outlines the responsibilities of institutions, animal ethics committees (AECs) and investigators in situations involving

a) the institutions that use an AEC established by another institution,

b) the investigators who do not have direct access to the institutional AEC and use the AEC established by the institution,

c) projects involving more than one institution or the AEC (or both), and

d) projects conducted by the South African investigators and institutions in other countries.

5.7.2 Governing principles

Each person involved in the care and use of animals for scientific purposes shall consider the governing principles in clause 4 when applying this standard to their specific circumstance; in particular:

a) the institutions, AECs, and people involved in any aspect of the care and use of animals for scientific purposes shall be aware of and accept their responsibilities, and act in accordance with the standard (see 4.7.4.1);

b) all activities, including projects, which involve the care and use of animals for scientific purposes shall

   1) be subjected to ethical review, approval and monitoring by the AEC,

   2) commence only after approval has been granted by the AEC,

   3) be conducted in accordance with the AEC approval, and

   4) cease if approval from the AEC is suspended or withdrawn or period of said approval ends (see 4.7.4.2).

5.7.3 The institutions and the investigators that use the external AEC

5.7.3.1 General

5.7.3.1.1 The institution that has established the AEC ("the host institution") may be approached by another institution ("the second institution") seeking to access the external AEC or share the AEC.

5.7.3.1.2 All institutions shall have a policy on the use of animals, whether they have the AEC or not. The policy shall be approved by the host institution’s executive, prior to any protocols being reviewed. It is up to the second institution’s executive to ensure that the approved policy is uniformly applied.

5.7.3.1.3 The host institution may also be approached by investigators who require the AEC approval for the care and use of animals for scientific purposes but who lack direct access to an AEC. In such cases, the host institution should consult with their AEC before they accept oversight of the care and use of animals for scientific purposes conducted on behalf of the second institution or investigator.

5.7.3.2 Institutions that use the AEC that has been established by another institution

When an institution uses the AEC that has been established by another institution, such use shall be based on a formal agreement that has been developed in consultation with the AEC. The agreement shall include

a) procedures for ensuring that the second institution can meet its responsibilities regarding the AEC, as outlined in 5.2,

b) procedures for communication between the AEC and the second institution, including governance and reporting,

c) an undertaking by the second institution that their investigators and other relevant personnel will abide by the directions of the AEC,

d) an undertaking by the second institution to abide by the AEC's policies and procedures regarding non-compliance, and

e) the circumstances under which either the institution may withdraw from the agreement.

5.7.3.3 The investigators who do not have direct access to the institutional AEC and use the AEC established by an institution

When the investigator who does not have direct access to the institutional AEC uses the AEC established by the institution, such use shall be based on a formal agreement that has been developed in consultation with the AEC. The agreement shall include

a) procedures for communication between the AEC and the investigator,

b) an undertaking by the investigator that they will abide by the directions of the AEC,

c) an undertaking by the investigator to abide by the AEC's policies and procedures regarding non-conformance, and

d) the circumstances under which either party may withdraw from the agreement.

5.7.4 Projects involving more than one institution or AEC (or both)

5.7.4.1 Responsibilities of institutions and animal ethics committees

5.7.4.1.1 The institutions shall ensure that projects involving investigators from more than one institution, or the care and use of animals at more than one institution, are approved and monitored by all of the responsible AEC(s). Procedures shall be developed and implemented to ensure that:

a) All parties involved are aware of, and can meet, their respective responsibilities under the requirements of the standard.

b) A project does not commence before each AEC approves, or the delegate AEC approves (see 5.7.4.1.2), activities to be conducted by members of its institution. Each AEC should be responsible for approval and monitoring of animal care and use that occurs at the institution for which it acts.

c) All the responsible AEC(s) are aware of all aspects of the proposed use of animals, and consider the cumulative effects on the well-being of the animals involved.

d) Prior to study commencement, the AECs involved should specify who has authority to undertake an inspection of the study. This will include inspection of all phases of the project. These agreed

inspectors can inspect the animals so that all phases of the project are monitored, including any animal transport between sites, and at any of the research or study sites, including those at either institution.

e) Clear communication channels are established between all the AECs and all the investigators.

f) The institution shall make provision in its policy to accept complains, requests for investigation or whistle blowing from the AEC or members of the other institution.

5.7.4.1.2 The institutions may agree to one AEC (the delegate AEC) approving the entire project, provided that all institutions involved agree to delegate the responsibility for decision making to, and support the necessary actions of, that AEC.

5.7.4.1.3 Arrangements between the two institutions should be as a formal agreement. The institutions should avoid unnecessary duplication of processes.

5.7.4.1.4 Arrangements should include mechanisms for reporting non-compliant activities between institutions and AECs.

5.7.4.2 Responsibilities of the investigators

The investigators shall notify the AEC of their institution, in writing if they are involved in collaborative animal studies at another institution, or if using animal products or tissues from another animal study (current or historic) performed at another institution, or if they are named in an application to the AEC of another institution (see 5.5.3.2.4).

5.7.5 Projects conducted by the South African investigators and institutions in other countries

5.7.5.1 Responsibilities of the institutions

5.7.5.1.1 The institutions should have procedures in place to ensure that, as a minimum, projects conducted on behalf of the institution in other countries

a) comply with the governing principles of this standard, and

b) are not conducted in other countries as a mechanism of avoiding compliance with this standard.

5.7.5.1.2 The institutions that operate facilities that use animals for scientific purposes in other countries should ensure that projects conducted at those facilities comply with the principles of this standard as a minimum, provided that such compliance does not breach relevant local legislation.

5.7.5.2 Responsibilities of the AEC

5.7.5.2.1 When considering approval for a project to be conducted in another country, the AEC may accept approval granted by the local AEC or its equivalent in that country if it is satisfied that outcomes would be equivalent to those expected through application of this standard.

5.7.5.2.2 The AEC shall ensure that animal care and use in the other country is adequately monitored. The AEC may appoint an agent or delegate to conduct the monitoring and inspection on its behalf.

5.7.5.3 Responsibilities of the investigators

5.7.5.3.1 The investigators responsible for a project conducted in another country should, as a minimum, ensure that

a) the project complies with the governing principles of this standard, and

b) the project is not conducted in another country as a mechanism of avoiding compliance with this standard.

5.7.5.3.2 The investigators who plan to use animals in another country shall obtain approval from their institutional AEC for such use. Investigators shall provide the AEC with advice on how the proposed project can meet the principles of this standard, taking into account compliance with local requirements.

5.8 Responsibilities of the institutions when developing the AEC application form

5.8.1 General

This clause outlines the responsibilities of institutions when developing documentation for application to the AEC for approval for the care and use of animals for scientific purposes.

5.8.2 Governing principles

Each person involved in the care and use of animals for scientific purposes shall consider the governing principles in clause 4 when applying this standard to their specific circumstance; in particular:

a) The care and use of animals for scientific purposes shall be subject to ethical review (see clause 4.2.2).

b) A judgement as to whether a proposed use of animals is ethically acceptable shall be based on information that demonstrates the principles in 4.2.1, and shall balance whether the potential effects on the well-being of the animals involved is justified by the potential benefits (see 4.2.3).

c) The AEC shall be satisfied that there is sufficient evidence to support a case that the proposed use of animals is justified (see 4.2.2).

d) All activities, including projects and procedures performed by the research animal facility staff that involve the care and use of animals for scientific purposes shall

1) be subject to ethical review, approval and monitoring by the AEC,

2) commence only after approval has been granted by the AEC,

3) be conducted in accordance with the AEC approval, and

4) cease if approval from the AEC is suspended or withdrawn or period of said approval ends (see 4.7.4.2).

5.8.3 Responsibilities

5.8.3.1 General

5.8.3.1.1 The institutions that establish the AEC shall, in consultation with the AEC, develop documentation (an application form) for applications for AEC approval to commence a project or activity and to amend an approved project or activity (see 5.3.3.5.10). The institution will determine the style of the application form (for example, electronic, web based, paper based).

5.8.3.1.2 The institutions shall ensure that the design of the application form allows for the provision of information required by the AEC to assess the ethical acceptability of the proposed use of animals (see 4.2.3 and 5.4.3.2.2).

5.8.3.1.3 The institutions shall ensure that procedures for applying to the AEC include a requirement for the use of plain language (understandable by laypersons) in the application, so that all AEC members are provided with sufficient information to participate effectively in the assessment of the application.

5.8.3.2 Information to be provided to the AEC for a project (see annex R)

The application form to commence a project shall allow the applicant to provide the following information, as appropriate for the circumstances:

a) The aims or hypothesis (or both) of the project. This may include an outline of how the project relates to an overall programme of work.

b) The potential benefits of the outcomes, and the evidence that supports the use of animals. For teaching projects, justification shall include an outline of how the attainment of educational outcomes will be assessed.

c) Details of why the use of animals is essential to achieve all the stated aims, potential alternatives that are available to replace the use of animals in all or part of the project, and why these alternatives are not suitable.

d) Information to support the case for ethical acceptability of the proposed use of animals, based on whether such use demonstrates the principles of this standard, and balancing whether the potential effects on the well-being of the animals involved is justified by the potential benefits.

e) Particular justification for activities that involve:

   1) severe compromise to animal well-being, and for which the four Rs cannot be fully applied for the project to proceed, including:

      i) pain, suffering, distress and lasting harm, including where the planned endpoints will allow severe adverse effects to occur (see 4.5.3 and 6.3.3.2.5 to 6.3.3.2.6 (inclusive))

      ii) death as the endpoint (see 4.5.4)

      iii) reuse and repeated use of animals (see 4.7.2.2 to 4.7.2.4 (inclusive) and 5.4.3.2.13)

      iv) prolonged restraint or confinement (see 10.6.3.6.3)

   2) use of non-human primates

      NOTE Using animals only when it is justified (see 4.2.1(a) and 4.3.1 to 4.3.2 (inclusive)).

f) An overview of how the project is designed in relation to its aims.

g) Details of animals:

   1) species, strain or breed, sex and age chosen, and the reason for this choice; and

   2) source of animals

      NOTE Applying high standards of scientific integrity (see 4.2.1(d) and 4.6.1 to 4.6.3 (inclusive)).

h) A clear description of the steps taken to consider and apply the four Rs.

i) The number of animals required and the justification for this number. Where appropriate, information shall be provided on:

   1) experimental design and statistical considerations, including statistical sample size calculations,

   2) for teaching projects, the ratio of students to animals, and the number of times that each animal will be used in each class, or handled per day or per week (or any two or all of these)

j) Opportunities for sharing of tissues and other biological material from animals being killed.

NOTE Applying the four Rs (see 4.2.1(e) and 4.7.1.1 to 4.7.3.6 (inclusive)).

k) Details of housing, husbandry and care of the animals, as well as environmental enrichment.

l) Details of the locations where animals will be housed and where procedures will be conducted.

m) Details and justification for care and management of animals that does not accord with current best practice.

NOTE Supporting the well-being of animals (see 4.2.1(b) and 4.4.1 to 4.4.2 (inclusive)).

n) Assessment of the potential adverse impact on animal well-being for the duration of the project, including:

   1) A step-by-step description of what will happen to each animal, or group of animals, for the duration of the project, including provisions for the animal at the conclusion of their use.

   2) Where applicable, procedures that apply to breeding programs that are integral to a project (such as the creation of a new line of animals, including genetically modified or cloned animals (see 5.5.3.4.4.1 to 5.5.3.4.4.2 (inclusive)), or that are integral to the maintenance of a line of animals in a facility (see 5.6.4.2(a)).

   3) Identification of known and potential causes of adverse impacts on the well-being of an animal and how such impacts will be avoided or minimized. Experimental and non-experimental factors shall be addressed (see 6.3.2.1 to 6.3.3.2.6 (inclusive)).

   4) Details of how all procedures (including euthanasia) will be performed, including SOPs and materials and methods.

o) Details of how the well-being of animals will be monitored and assessed throughout the project, the frequency of monitoring and assessment (including record keeping thereof), the actions to be taken if problems are identified, and the criteria for intervention points and humane endpoints (see 6.3.3.3.1 to 6.3.3.5.1 (inclusive)).

NOTE Avoiding or minimizing harm, including pain, suffering, distress and lasting harm, to animals (see 4.2.1(c) and 4.5.1 to 4.5.4 (inclusive)).

p) Identification of the person with ultimate responsibility for the conduct of the project or the care of the animals (or both) (see 5.5.3.1.6 and 5.6.3.1.3).

q) The name of the project, the people involved and their responsibilities.

r) The competence and necessary authorization with the relevant national council (see foreword), of people for all procedures they will undertake using animals, and details of their supervision by a person who is competent to perform the procedures.

s) Assurance that adequate resources, including human resources, will be available for the conduct of the project.

t) Details of any participation of staff from other institutions, and if and how the facilities of another institution will be used (see 5.5.3.2.4).

u) Any actual or potential interest, including any financial interest or other relationship or affiliation, that may affect judgements and decisions regarding the well-being of the animals involved, shall be declared.

v) Any additional administrative details as required by the institution and the AEC, for example, details of collaborations, permits and licences, certification of the biological status of the animals, and work health and safety considerations.

w) A declaration by the responsible investigator(s) stating that they and all others involved in the project are familiar, and will comply, with the requirements of this standard and all relevant legislation, and providing assurance that adequate resources will be available to undertake the project.

x) All permits and licenses required by national or provincial departments shall be submitted with the AEC prior to initiation of a project. This shall include indications of permission to use scheduled medication as defined by the relevant national legislation (see foreword).

NOTE Knowing and accepting responsibilities (see 4.2.1(d) and 4.7.4.1 to 4.7.4.2 (inclusive)).

5.8.3.3 Information to be provided to the AEC for activities associated with the care and management of animals in facilities

The application to commence activities associated with the care and management of animals in facilities should include the information outlined in 5.8.3.2, as appropriate for the circumstance.

5.8.3.4 Information to be provided to an animal ethics committee for an amendment to an approved project or activity

The application for an amendment (see 5.3.3.5.4) to an approved project or activity should include the information outlined in 5.8.3.2, where relevant.

5.9 Responsibilities of the attending veterinarian and veterinary care

5.9.1 General

This clause outlines the responsibilities of the attending veterinarian and veterinary care in institutions.

5.9.2 Governing principles

5.9.2.1 Accepted international standards for veterinary oversight and care shall be followed (AAALAC International 2016; Committee for the Update of the Guide for the Care and Use of Laboratory Animals 2011; European Commission 2010 and the World Organisation for Animal Health: Terrestrial Animal Health Code, 2011).

5.9.2.2 Each institution that uses animals for scientific purposes should have an attending veterinarian with expertise in laboratory animal medicine, charged with advisory duties in relation to the well-being and treatment of animals. The role of the attending veterinarian extends beyond advice on disease or health issues, and is an integral part of the development of continued improvement of scientific practices, in particular with respect to refinements in model design, clinical monitoring, and a culture of care.

5.9.3 Responsibilities

5.9.3.1 The animal care and use programme is the collaborative responsibility of, the attending veterinarian, the AEC and where necessary the institutional official responsible for the animal care and use programme, who has the authority for resource allocation and planning. The animal care and use programme means the policies, procedures, standards, organizational structure, staffing, facilities and practices put into place by an institution to achieve the humane care and use of animals throughout the institution.

5.9.3.2 The attending veterinarian is responsible for the well-being and clinical care of animals used for scientific purposes. This responsibility extends to monitoring and promoting animal well-being at all times during animal use, and during all phases of the animal's life. It is expected that the programme of veterinary care will uphold the highest standards of care and ethics. The veterinarian shall have sufficient authority provided by the institution, to treat an animal and institute appropriate measures to relieve severe pain or distress, including euthanasia. A veterinarian should have the ultimate decision making ability of when to euthanize an animal.

5.9.3.3 In fulfilling these duties in a research environment, the attending veterinarian should interact collaboratively with the research team (for example, the principal investigator) when making critical decisions regarding animal health and welfare, during project planning and implementation. The attending veterinarian shall have adequate resources to manage the overall programme of veterinary care. Other qualified individuals may assume some of the roles and responsibilities of the attending veterinarian, as appropriate, under the oversight of the attending veterinarian in accordance with the relevant national legislation (see foreword).

5.9.3.4 Several factors will determine whether full-time, part-time or consultative veterinary services are needed at a given institution. If a full-time veterinarian is not available on-site, a consulting or part-time veterinarian should be available and conduct visits at intervals appropriate to the animal care and use programme's needs. In such instances, there shall be an individual with assigned responsibility for the management of daily animal care and use and facility management. While institutions with large programmes may employ multiple veterinarians, the management of veterinary medicine, animal care and facility operations by a single administrative unit is often an efficient mechanism to administer all aspects of the animal care and use programme.

5.9.3.5 Important aspects of the role of the attending veterinarian and the programme of veterinary care should include the following:

a) veterinarians providing clinical or programme oversight (or both) and support shall have experience, training, and expertise necessary to appropriately evaluate the health and well-being of the species used, in the context of the animal use being carried out by the institution;

b) the attending veterinarian shall have access to all animals;

c) there shall be timely provision of veterinary medical care, and emergency veterinary care shall be available at all times, including after work hours, on weekends, and on holidays;

d) the attending veterinarian shall have oversight of additional aspects of the veterinary care programme, such as preventative medicine and health surveillance, medical treatment, establishment of sedation, anaesthetic and analgesic guidelines, handling, and immobilization, and should have oversight of other related aspects such as housing and husbandry;

e) the attending veterinarian should provide guidance and oversight to surgery programmes and perioperative care; and

f) professional veterinary staff should remain knowledgeable about the latest practices and procedures to ensure that high quality care is provided to animals.

5.9.3.6 In addition, the attending veterinarian has further responsibilities related to his or her role on the AEC. For example:

a) there should be regular, clear communication between the attending veterinarian and the AEC, and animal programme needs should be regularly and clearly communicated to the institutional official by the attending veterinarian;

b) the veterinarian should have input in protocol review, the development of study removal criteria, and responsible conduct of research activities;

c) the veterinarian or individuals providing care to animals (or all of these) should understand the potential for adverse clinical complications that may arise from experimental procedures; and

d) the AEC, in association with the attending veterinarian, has the responsibility for ensuring that personnel performing surgical, other clinical or veterinary procedures are appropriately qualified, trained and competent in the procedures to be performed.

5.9.4 Summary of main tasks

5.9.4.1 Establishing a programme of veterinary care and collaboration with the AEC

A programme of veterinary care and collaboration with the AEC shall be established to deliver the following:

a) provision of advice and veterinary services regarding choice of species and strains, transport, import and export of animals;

b) provision of advice regarding animal acquisition, husbandry, housing and care;

c) surveillance of the health status, prevention, detection, treatment and control of diseases (including zoonoses) and disaster planning in case of outbreaks;

d) contribution to the work of the AEC on matters of animal health and welfare and the implementation of the four Rs;

e) input and advice to the researchers, the person(s) responsible for the project and the AEC on animal use models, experimental design (as appropriate), implementation of the four Rs and severity assessment of procedures;

f) recognition and management of adverse events impacting the health or welfare of animals, whether associated with an experimental protocol or not;

g) provision of advice and recommendations for non-surgical and surgical interventions;

h) provision of advice and guidance for the anaesthesia, analgesia, post-operative care and alleviation of pain, suffering and distress in relation to experimental protocols;

i) assessment of the well-being of animals and recognition of severity classification;

j) establishment and maintenance of adequate clinical observation sheets;

k) keeping of accurate veterinary records;

l) provision of advice and guidelines regarding implementation of humane endpoints and euthanasia practices;

m) veterinary examination and advice and decision taking regarding:

1) keeping an animal alive at the end of procedures;

2) animals taken from the wild that are found in poor health;

3) reuse of animals and the related aspects to be taken into account;

4) re-homing of animals and the related aspects to be taken into account, as specified in the relevant national legislation (see foreword);

5) environmental impact of the capture or release (or both) of wild animals.

NOTE A veterinarian may provide useful expert input on project evaluation.

5.9.4.2 Involvement in training/supervision/assessment

A veterinarian may also provide useful expert input in evaluating whether an adequate training programme is in place regarding

a) embedding of a culture of care in the overall training programme,

b) handling and care of laboratory animals,

c) handling and care during procedures,

d) clinical observation and their correct recording,

e) non-surgical and surgical procedures in the species concerned, and

f) euthanasia practices.

5.9.4.3 Training of attending veterinarians

5.9.4.3.1 Veterinarians have a strong background in animal health, disease, welfare and hygiene. However, the field of Laboratory Animal Medicine and Science represents a focused area of veterinary expertise and additional post-graduate veterinary training may be needed to optimally fulfil the roles and responsibilities of the attending veterinarian.

5.9.4.3.2 The general appreciation of animal care, health and management; the recognition of pain, suffering and distress; and anaesthesia, analgesia and surgery are all part of normal professional veterinary training. Species-specific specialization (for example, non-human primates, avian, fish, cephalopods) can be dealt with as and when required following a gap analysis and as part of Continuing Professional Development (CPD).

5.9.4.3.3 Core competencies should include appropriate knowledge of the legal environment.

5.9.4.3.4 Veterinarians should have adequate core competencies that are specific to the relevant species and related practices, for example, in the field of Laboratory Animal Medicine and Science, including relevant competencies that are not included in the normal veterinary curriculum.

5.9.4.3.5 The exact definition of additional training needs will depend on the activities of the establishment (for example, species involved and type of activities, for example, the breeder or supplier versus the user). Competencies should be tailored to the needs of the establishment and the veterinarian.

5.9.4.3.6 Core competences should preferably be gained prior to starting a attending veterinarian assignment, or as soon as possible thereafter.

5.9.4.3.7 The attending veterinarian should ideally complete additional training in the following:

a) national legislation pertaining to animal use for scientific purposes;

b) ethics, animal welfare and the four Rs; and

c) the design of procedures and projects.

5.10 Responsibilities for education, training, supervision and competence of personnel in institutions

5.10.1 General

This clause outlines the responsibilities on education, training, supervision and competence of personnel in institutions. Performing procedures or working with or on animals remains subject to authorization or registration with the relevant national council (see foreword).

5.10.2 Governing principles

5.10.2.1 Each animal facility and study area should have sufficient competent personnel on-site.

5.10.2.2 Education and training should facilitate and assure the competence of all persons involved in the care, use and breeding of all animals used for scientific procedures.

5.10.2.3 The requirements for education, training and competence apply to all animal species, to all types of scientific institutions or facilities and to all types of housing enclosure or study area, including laboratory-housed, domestic, agricultural, feral and wildlife animal populations.

5.10.2.4 All personnel, including researchers, students and staff, should be adequately educated and trained before they perform any of the following:

a) carrying out procedures on animals, including capture or restraint;

b) designing procedures or projects (or both);

c) taking care of animals; or

d) killing animals.

5.10.2.5 Education and training should include consideration of the training, supervision, competence assessment and continuing training requirements of persons carrying out procedures, taking care of animals, killing animals and of those responsible for the design of procedures and projects.

5.10.2.6 Persons who design procedures or projects (or all of these) should have received instruction in a scientific discipline relevant to the work being undertaken and should have species-specific knowledge.

5.10.2.7 Persons carrying out procedures on animals (including capture or restraint), taking care of animals, or killing animals, should be supervised in the performance of their tasks until they have demonstrated the requisite competence.

5.10.2.8 Education and training should be subject to quality assurance oversight.

5.10.3 Subject matter for education and training

5.10.3.1 Philosophy of animal use

Education and training on the philosophy of animal use shall include the following points:

a) the candidate should be introduced to the philosophy of animal use for scientific purposes;

b) the aim should be to introduce an unbiased overview of the philosophical arguments for and against animal use for research, teaching, testing and other scientific purposes;

c) candidates should be introduced to (though discussions should not be limited to):

1) the definitions of ethics and morality;

2) different philosophical worldviews as this relates to animal use;

3) concepts in ethics of research on people;

4) constitutional rights of citizens;

5) legal objects versus legal persons;

6) animal sentience;

7) animal rights, animal freedoms and animal welfare;

8) moral rights versus legal rights;

9) benefits and weaknesses of animal models and non-animal models in research;

10) legislation protecting animals versus legislation mandating animal testing;

11) the use of animals for scientific purposes versus other uses of animals in society;

12) the human-animal relationship;

13) the intrinsic value of life; and

14) the concept of the dignity of animals.

d) overall this discussion and debate should allow a person to develop their own views and opinions of the use of animals for scientific purposes; and

e) other topics that may be discussed include culture of care and compassion fatigue.

5.10.3.2 The four Rs

5.10.3.2.1 The practical application of the core ethical principles of the four Rs should be discussed in detail (see 4.7), in order to maximize useful outcomes with the least possible pain, fear, suffering, distress or lasting harm to animals, i.e. the harm-benefit analysis.

5.10.3.2.2 Candidates should be able to discuss and apply the four Rs critically in projects.

5.10.3.2.3 The design of procedures and projects should be covered where appropriate.

5.10.3.3 National standards and legislation

5.10.3.3.1 This should introduce the keys concepts contained in this standard, including but not limited to the responsibilities of researchers and teachers, the responsibilities of institutions and responsibilities of the AEC.

5.10.3.3.2 Focus shall be given to the AEC, composition and functioning of committees, management of conflicts of interest, and the importance of robust debate around animal well-being versus scientific outcomes, such as the harms-benefit analysis.

5.10.3.3.3 Candidates should be taken through actual case studies so that they can be directly inducted into the ethical debate and practical review of the application of the four Rs.

5.10.3.3.4 The relevant national legislation (see foreword) that governs the responsible care and use of animals for scientific purposes should be reviewed, including associated regulations, rules and policies.

5.10.3.4 Species-specific housing, care, biology and procedures

5.10.3.4.1 Species-specific housing, care, biology and procedures training will be dependent on the species used.

5.10.3.4.2 The training should include all aspects of housing and care of the animal species in question, including but not limited to:

a) handling and restraint;

b) species-specific normal and abnormal behaviour, physiology and anatomy;

c) recognition of species-specific distress, pain and suffering; husbandry and environmental enrichment;

d) breeding;

e) genetics and genetic alteration;

f) hygiene control;

g) basic disease information and animal health management;

h) practical procedures;

i) sampling;

j) dosing;

k) anaesthesia, sedation, analgesia and euthanasia requirements; and

l) the use of species-appropriate humane endpoints.

5.10.3.4.3 Appropriate practical training under the supervision of a competent teacher shall support this training until practical competence has been attained and maintained in all relevant practical procedures.

5.10.3.5 Work-integrated training

5.10.3.5.1 A person working within the field should be trained to competently undertake activities specific to their field. This may be achieved in a number of different ways, for example, by obtaining a formal qualification, attending training courses or CPD events that include practical training, or undertaking work shadowing in the workplace.

5.10.3.5.2 For work shadowing, the candidate can spend time at a research animal facility or other study area (for example, wildlife area or farm), gaining hands-experience with the species in question by working directly with experienced and competent persons.

5.10.3.5.3 Veterinarians can shadow veterinarians, while laboratory animal technologists, veterinary nurses, animal caretakers and researchers can shadow veterinarians, laboratory animal technologists or veterinary nurses.

5.10.3.5.4 Supervisors are a key aspect to this training. Supervisors should be veterinarians, laboratory animal technologists or veterinary nurses with appropriate and sufficient experience. In recognition of the potential of some research animal facilities being staffed with potential mentors with limited experience (for example, less than five years), additional external mentoring should be provided by experienced mentors who should be available to assist as a consultant (even if telephonically).

5.10.3.5.5 In the case of veterinarians, who may be unable to work directly with an experienced veterinary mentor, it is suggested that they work with an experienced laboratory animal technologist (with at least five years of experience), with an experienced laboratory animal veterinarian available as a consultant (even if telephonically). The idea behind veterinary shadowing will be to supplement general veterinary training and experience with context-specific application of veterinary and scientific principles.

5.10.4 Duration of education and training

5.10.4.1 In general, it is advised that at least a week (i.e. 30 h to 40 h) of education and training is given.

5.10.4.2 Assessment procedures should include written tests and also observation where appropriate. Assessments should provide confidence that the trainee has achieved a suitable level of understanding to meet the learning criteria.

5.10.4.3 Supervision after the initial period of training is essential to ensure that full working competence is obtained and maintained. Periods of supervision may vary from around 3 months for animal caretakers to 12 months for trainee laboratory animal technologists.

5.10.4.4 Duration of the supervision period and time taken until competence is attained will vary, for example, due to the frequency of the task being performed, technical complexity, and ability of the individual. It is, therefore, not desirable to specify time limits for teaching or supervision periods.

5.10.5 Supervision and competence

5.10.5.1 Requirements for education and training, as well as the requirements for obtaining, maintaining and demonstrating competence, should be considered on the basis of the following:

a) Education and training alone do not deliver competence.

b) A period of supervision will generally be necessary, to re-enforce understanding and to ensure the tasks or duties or procedures are conducted to an appropriate standard, with interventions as required by the supervisor(s) to ensure this is attained.

c) Only after individuals have been assessed as competent, should they work without supervision. By this time, those deemed competent should have attained a deeper understanding of the task.

d) Competence should be subject to periodic review.

5.10.5.2 Training should be a considered as a continuous process, through the initial training, to a period of working under supervision until such time as competence is attained.

5.10.5.3 When there is a likelihood of causing pain, suffering, distress or lasting harm, the relevant training should be completed prior to working under supervision. If this is not the case, the trainee could begin working under supervision before the training is satisfactorily completed.

5.10.5.4 The assessment of practical skills should ensure that the trainee could proceed to working under supervision with no increased risk to animal welfare. The proficiency in skills will be developed during working under supervision. The period and level of supervision will vary according to the complexity of the task, its frequency and previous experience of the trainee.

5.10.5.5 The responsibility for the correct performance of the task remains with the supervisor in all cases until such time the training is completed and the requisite competence demonstrated.

5.10.5.6 Duration of the supervision period and time taken until competence is attained will vary, for example, due to the frequency of the task being performed, technical complexity, and ability of the individual.

5.10.5.7 The objective of the initial training is the attainment of basic knowledge and understanding, with the concept that a deeper understanding of the knowledge base, as well as proficiency in skills, should have developed and be expected by the time competence is assessed.

5.10.5.8 Where procedures are performed intermittently or rarely, or individuals have not performed procedures for some time, consideration should be given to the provision of additional supervision.

5.10.5.9 For assessment of competence, the assessor should observe and evaluate the trainee performing the procedures to assess practical competence.

5.10.5.10 A mechanism should be in place to ensure that incompetence or poor practice in any person is recognised and reported to allow appropriate remedial action to be taken.

5.10.5.11 Review of and maintenance of competence should be considered as an on-going process and there should be oversight to ensure that acceptable standards are maintained.

5.10.5.12 Individuals should maintain competence through a process of continuing education CPD. CPD is intended to ensure that all persons involved in the care and use of animals remain competent and up-to date on new developments in the field.

5.10.5.13 Further information regarding recommended best practice training structures, with associated learning outcomes and assessment criteria, are given for reference in annex T.

6 Animal well-being

6.1 General

6.1.1 This clause applies to all species of animals used for scientific purposes, and to all activities and situations involving their care and use. It outlines the principles for supporting and safeguarding the well-being of animals used in terms of the animal's lifetime experience. Information in this clause outlines how to approach supporting and safeguarding the well-being of animals and provides information on safeguarding the well-being of animals during the conduct of specific procedures and at the conclusion of their use.

6.1.2 Information provided in this clauses is based on the assumption that approval has been obtained from the AEC before any activity, including projects, commences (see 4.7.4.2). The necessity and requirements for the AEC approval are addressed in 5.4.

6.2 Governing principles

Each person involved in the care and use of animals for scientific purposes shall consider the governing principles in clause 4 when applying this standard to their specific circumstance; in particular:

a) The well-being of animals used for scientific purposes shall be considered in terms of the cumulative effects of the animal's lifetime experience. At all stages of the care and use of an animal, measures should be taken to ensure that the animal's environment and management are appropriate for the species and the individual animal, and support the animal's well-being (see 4.4.1).

b) Animals have a capacity to experience pain, suffering, distress and lasting harm, even though they may perceive and respond to circumstances differently from humans. Pain, suffering, distress and lasting harm may be difficult to evaluate in animals. Unless there is evidence to the contrary, it shall be assumed that procedures and conditions that would cause pain, suffering, distress and lasting harm in humans cause pain, suffering, distress and lasting harm in animals. Decisions regarding the possible impact of procedures or conditions on an animal's well-being shall be made in consideration of an animal's capacity to experience pain, suffering, distress and lasting harm (see 4.5.1).

c) All possible steps shall be taken at all times to safeguard the well-being of animals by avoiding or minimising harm, including pain, suffering, distress and lasting harm, to the animals (see 4.5.2).

d) The development of strategies to support and safeguard animal well-being shall include the application of high standards of scientific integrity (see 4.6.1 to 4.6.3 (inclusive)), and the application of replacement, reduction, refinement and responsibility (the four Rs) (see 4.7.1.1 to 4.7.3.6 (inclusive)).

6.3 Strategies to support and safeguard animal well-being

6.3.1 General

The planning and conduct of activities involving the care and use of animals shall support and safeguard animal well-being. Steps include

a) identifying known and potential causes of adverse impact on animal well-being, taking into consideration both intended and unforeseen consequences,

b) taking steps to avoid or minimize adverse impacts, including setting intervention points and humane endpoints, and monitoring animals,

c) reviewing the effectiveness of strategies to support and safeguard animal well-being,

d) implementing changes to strategies to ensure the on-going support and safeguarding of animal well-being, and

e) ensuring that all relevant people are aware of and accept their responsibilities regarding the well-being of the animals.

6.3.2 Identify known and potential causes of adverse impacts on animal well-being

6.3.2.1 Circumstances with the potential to have an adverse impact on the well-being of an animal shall be identified. Experimental and non-experimental causes shall be considered, including acquisition and breeding, capture, transport, housing and care, social and physical environment, handling, restraint, sample collection, non-surgical procedures, anaesthesia, surgical procedures, genetic modification, disease induction, humane killing and provisions for the animal at the conclusion of their use.

6.3.2.2 In each instance, factors that might contribute to the level and duration of pain, suffering, distress and lasting harm, and the risk of such occurrences, shall be considered and assessed, taking into account the predicted likelihood and consequences.

6.3.2.3 If the potential impact on the animal, or the validity and efficacy of criteria for intervention to minimize pain, suffering, distress and lasting harm, cannot be predicted on the basis of available evidence, the incorporation of a pilot study into the design of the project should be considered.

6.3.3 Take steps to avoid or minimize adverse impacts on animal well-being

6.3.3.1 Support the animals' well-being

6.3.3.1.1 Animals shall be cared for and managed so that species-specific or strain-specific physiological and behavioural needs are met.

6.3.3.1.2 Practices and procedures used for the care and management of animals shall be appropriate for the situation, the species and strain of animal, weight, age and sex and the activities to be undertaken, and shall be based on current best practice. Where the requirements of a project or activity preclude or modify these conditions, special ethical consideration and specific AEC approval is required.

6.3.3.1.3 The living conditions in which animals are bred, held and used shall be checked daily (see 10.6.3.1.2).

6.3.3.1.4 Procedures shall be in place at all stages of animal transport (refer to all relevant legislation), supply, housing and care to ensure that a health status of the animals is maintained that safeguards animal well-being and meets the requirements of their proposed use (see10.2 to 10.6 (inclusive)).

6.3.3.1.5 Animals that are sourced, bred or held for scientific purposes shall be suitable for their proposed use, taking into account their biological characteristics; temperament, behavioural conditioning, microbiological and nutritional status, and general state of health (see 4.6.3). Where appropriate, the suitability of animals should be assessed before they are selected.

6.3.3.1.6 Assessment of animals (for example, well-being, suitability for purpose, health) shall be undertaken by a competent person, or under the direct supervision of a competent person registered in terms of the relevant national legislation (see foreword).

6.3.3.1.7 Animals should be acclimatized for a minimum of five days in the same housing or holding conditions as the experiment before they are used (see 10.5.1 to 10.5.2 (inclusive)). Unless acclimatization for a shorter period can be scientifically justified and is approved by the AEC.

6.3.3.1.8 For animals that normally live in social groups, social isolation or separation from a group shall be avoided unless specific justification is provided to, and approval is obtained from, the AEC (see 10.6.3.1, 11.6.3.1 and A.5.7).

6.3.3.1.9 Animals shall be identified.

6.3.3.2 Avoid or minimize pain, suffering, distress and lasting harm

6.3.3.2.1 Animals used shall be suited to the purpose of the project or activity (see 4.6.3), and their suitability and sustainability shall be assessed before they are used.

6.3.3.2.2 Scientific and educational methods used shall be in accordance with the four Rs.

6.3.3.2.3 Procedures, husbandry and care shall be performed competently, by people who are competent or by people under the direct supervision of a competent person in terms of the relevant legislation (see foreword).

6.3.3.2.4 Potential causes of pain, suffering, distress and lasting harm that are not part of the design of a project or activity should be eliminated or controlled to minimize the adverse impact on animal well-being and the risks to quality of data.

6.3.3.2.5 If pain, suffering, distress and lasting harm are predicted or unavoidable consequences of a project, methods for minimising such pain, suffering, distress and lasting harm shall be incorporated into the design of the project, including

a) establishing and implementing early intervention points and endpoints (see 6.3.3.4.1 to 6.3.3.5.3 (inclusive)),

b) monitoring animals to ensure that the planned endpoints are detected, and taking appropriate action (see 6.3.3.3.1 to 6.3.3.3.6 (inclusive)),

c) using pharmacological agents and non-pharmacological measures for avoiding and minimising pain, suffering, distress and lasting harm (see 6.4.6.1 to 6.4.6.8 (inclusive) and 6.4.8.1(c)), and

d) when pain is unavoidable the study shall include a pilot phase to indicate the unavoidable necessity of pain in the study design.

6.3.3.2.6 Where it is established that the aim(s) of the project involves animals experiencing pain, suffering, distress and lasting harm that will not be alleviated:

a) the planned endpoint of the project shall be as early as feasible to avoid or minimize pain, suffering, distress and lasting harm to the animals; and

b) the animals shall be monitored and assessed so that the planned endpoints are detected, and actions shall be taken in accordance with the AEC approval for the project.

6.3.3.3 Monitor animals and take appropriate action

6.3.3.3.1 The animals shall be monitored and assessed

a) by a competent person who is knowledgeable about the normal behaviour and signs of pain, suffering, distress and lasting harm for the species, or a person under the direct supervision of a competent person in terms of the relevant national legislation (see foreword),

b) with sufficient frequency at minimum twice daily to ensure that any harm, including pain, suffering, distress and lasting harm, is promptly detected and managed, and

c) in accordance with the AEC approval for the project or activity.

6.3.3.3.2 Methods for monitoring and assessment of animal well-being should include

a) the criteria that will be used to assess well-being,

b) the level and frequency of monitoring to ensure that any changes in an animal's condition are detected early,

c) the criteria that will be used to determine when action is required,

d) actions that will be taken so that adverse impacts on animal well-being, including predicted effects and unforeseen complications, are addressed rapidly and effectively,

e) the methods for recording observations, treatments and actions, and

f) flexibility to ensure a rapid and effective response to changes during the course of the project or activity.

6.3.3.3.3 Records of the monitoring and assessment of animal well-being shall be

a) sufficient to enable the AEC to verify that the well-being of animals has been monitored as agreed, and allow review and critical investigation of the cause(s) of and responses to unscheduled adverse events as a basis for future prevention strategies,

b) accessible to all people involved in the care of the animal, and

c) available for audit by the institution, the AEC and authorized external reviewers.

6.3.3.3.4 Prompt action shall be taken based on the monitoring and assessment of animals, in accordance with

a) institutional and the AEC policies and procedures (see 5.2.3.3(e)(3)), and

b) the intervention points and humane endpoints approved by the AEC for a project, or actions documented in procedures for animal care approved by the AEC.

6.3.3.3.5 Prompt action shall be taken in response to unexpected adverse events and emergencies, including alleviation of pain, suffering, distress and lasting harm, in accordance with institutional and AEC policies and procedures (see 5.2.3.3(e)(3)). Alleviation of pain, suffering, distress and lasting harm of a severity that was not anticipated in an approved project or activity shall take precedence over an individual animal reaching the planned endpoint of the project or activity, or the continuation or completion of the project or activity. If necessary, animals shall be killed humanely without delay (see 5.5.3.4.1.7(i), 5.6.3.1.3 and 5.9.3.2)).

6.3.3.3.6 When an animal dies unexpectedly, or is humanely killed due to unforeseen complications, a necropsy should be performed by a competent person (see 5.2.3.3(e)(3)) as certified in terms of the relevant national legislation (see foreword).

6.3.3.4 Set intervention points and experimental humane endpoints

6.3.3.4.1 If pain, suffering, distress and lasting harm are predicted or unavoidable consequences of a project, validated criteria that are appropriate for the species and the nature and time course of the predicted effects shall be established to identify

a) the earliest time point at which data can be obtained and the study completed (experimental endpoint(s)), see 4.5,

b) when intervention is necessary to minimize pain, suffering, distress and lasting harm (intervention point(s)), and

c) when the animal should be humanely killed, regardless of whether the aims of the study have been achieved (humane endpoint(s)).

6.3.3.4.2 Intervention points and endpoints shall be applied as early as feasible and ensure that

a) the duration and extent of pain, suffering, distress and lasting harm are minimized, and

b) valid data are obtained at the earliest time point before or following the onset of pain, suffering, distress and lasting harm (refer to annex P).

6.3.3.5 Endpoint evaluations (refer to annex P)

6.3.3.5.1 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 shall be put to death by recognized euthanasia methods for animal welfare reasons.

6.3.3.5.2 Death as an endpoint is generally ethically unacceptable.

6.3.3.5.3 Death as an endpoint shall be replaced with early experimental and humane endpoints whenever possible. Where death as an endpoint is essential for the aim(s) of the project and cannot be avoided:

a) The project shall be designed to minimize the number of animals that will die.

b) Steps to avoid or minimize pain, suffering, distress and lasting harm, including early experimental and humane endpoints, shall be considered, implemented and reviewed at all stages of the project.

c) All animals found in a moribund state shall be put to death by recognized euthanasia methods in order to alleviate suffering and in addition these animals may give unreliable research data due to multiple organ failure. Endpoints earlier than a moribund condition shall always be used.

6.3.4 Accept responsibilities

6.3.4.1 The persons responsible for the well-being of animals at any given time shall be clearly identified (see 5.2.3.5.5(a), 5.5.3.4.1.8(b) and 5.6.3.1.1).

6.3.4.2 When developing strategies for supporting and safeguarding animal well-being, the investigators and animal caretakers should

a) consult with all relevant people or (or all of these) groups responsible for the well-being of the animals,

b) clearly identify the person responsible for monitoring the animals,

c) ensure good communication and cooperation between all parties involved, and

d) consult relevant literature.

6.4 Specific procedures

6.4.1 General

This clause outlines how the well-being of animals may be supported and safeguarded during the conduct of specific procedures. This includes procedures used during the care and management of animals and procedures used during the conduct of approved projects. For species specific information, refer to the relevant annex for each species.

6.4.2 General requirements that apply to all procedures

Procedures shall

a) be appropriate for the species, life stage and the circumstances,

b) be in accordance with the current best practice in terms of the four Rs,

c) be compatible with the purpose and aims of the project or activity,

d) cause the least pain, suffering, distress and lasting harm, to the animals,

e) be performed competently, and by a person who is competent for the procedures, or when being trained, be under the direct supervision of a person who is competent to perform the procedures, and

f) where applicable, be in accordance with the relevant national legislation (see foreword).

6.4.3 Husbandry procedures

6.4.3.1 All husbandry procedures shall be performed competently, and by a person who is competent for the procedures, or when under training be under the direct supervision of a person who is competent to perform the procedures.

6.4.3.2 Routine husbandry procedures are not part of a project and include, for example, but not limited to, cleaning of cages, provision of food and water, breeding, weaning, enrichment of the environment, weighing, deworming, clinical observation, clipping coats and nails, and vaccinations (unless the procedure is being studied as part of the research experiment).

6.4.4 Identification of animals

6.4.4.1 Methods used to identify animals shall

a) Be appropriate for the species, life stage and the circumstances.

b) Be compatible with the purpose and aims of the project or activity.

c) Involve non-invasive methods whenever possible. The use of invasive methods shall conform with 6.4.2.

d) Cause the least pain, suffering, distress and lasting harm to the animals.

e) Be in accordance with the current best practice in terms of the four Rs.

f) Where applicable, be in accordance with the relevant national legislation (see foreword).

6.4.4.2 For additional information on identification of wildlife, see 11.10 and for other species refer to the relevant species specific annex in this standard.

6.4.5 Injections, blood sampling and non-surgical procedures

When performing injections, blood sampling and non-surgical procedures, procedures used shall

a) minimize the risk of an animal experiencing pain and distress; or developing complications(for example, tissue damage, infection, haemotoma, bleeding),

b) be performed under aseptic conditions if there is potential risk of infection, and

c) if the procedure involves the transplantation of cells or tissues, include management of the effects of tissue rejection and immunosuppression.

6.4.6 Anaesthesia, analgesia and sedation, and management of pain, suffering, distress and lasting harm

6.4.6.1 The use of local and general anaesthetics, analgesics and sedatives shall be considered as part of a plan to manage pain, suffering, distress and lasting harm, and such use should at least parallel their use in current veterinary or medical practice.

6.4.6.2 When anaesthetics, analgesics and sedatives are used, the choice of agent and its administration shall

a) be appropriate for the species, age, developmental stage and physiological status of the animal,

b) be compatible with the purpose and aims of the project or activity, and appropriate for the type of procedure, and

c) where applicable, be in accordance with the relevant national legislation (see foreword).

6.4.6.3 Unless there is evidence to the contrary, it shall be assumed that fetuses have comparable requirements for anaesthesia and analgesia as adult animals of the species. Approaches to avoid or minimize pain, suffering, distress and lasting harm in the fetus shall be designed accordingly.

6.4.6.4 Regardless of their mechanism of action, the effectiveness of all anaesthetics shall be monitored throughout anaesthesia.

6.4.6.5 When general anaesthesia is used, procedures shall conform with current veterinary or medical practice and ensure that

a) induction is smooth, with minimum distress to the animal,

b) the animal and the effectiveness of the anaesthetic are monitored throughout the procedure to maintain an adequate plane of anaesthesia, minimize physiological disturbances, and monitor and manage potential complications (for example, hypothermia, and cardiovascular and respiratory depression),

c) when the animal recovers from an anaesthetic, the animal is monitored and cared for to avoid and manage complications throughout the period of recovering from anesthesia (for example, airway

obstruction, hypothermia, cardiovascular and respiratory compromise, injury from uncoordinated movements or other animals), and

d) records are maintained of the use of anaesthetics and other drugs, monitoring of the animal, and the management of complications.

6.4.6.6 Animals that develop signs of pain, suffering and distress shall be treated promptly, in accordance with the intervention points and humane endpoints approved by the AEC, and the institutional and the AEC policies and procedures (see 5.2.3.3(e)(4) and 6.3.3.3.4 to 6.3.3.3.5 (inclusive)).

6.4.6.7 Neuromuscular blocking agents shall only be used in conjunction with adequate general anaesthesia or an appropriate surgical procedure that eliminates sensory awareness, except when species differences dictate otherwise. The animal shall be monitored to ensure that an adequate plane of anaesthesia is maintained or sensory awareness has been eliminated. Because the paralysis abolishes many criteria for assessing anaesthetic depth and pain perception (for example, character of respiration, and corneal and flexor withdrawal reflexes), continuous or frequent monitoring of physiological variables (for example, heart rate, blood pressure, pupil size, electroencephalogram), together with the effects on these of mild sensory stimuli, shall be used.

NOTE Neuromuscular blocking agents cannot be used as a sole method for euthanasia as the induced respiratory depression is extremely stressful.

6.4.6.8 Electroimmobilisation shall not be used as an alternative to analgesia or anaesthesia.

6.4.7 Surgical procedures

The well-being of animals that have undergone surgical procedures shall be supported and safeguarded by

a) Conducting surgical procedures under appropriate local or general (or both) anaesthesia. The well-being of the fetus or embryo shall be taken into account before conducting surgery on a pregnant female.

b) Using aseptic procedures if the animal is expected to recover from surgery.

c) Ensuring that all procedures and analgesia conform to the accepted standards in veterinary or medical practice, as appropriate for the procedure and circumstances.

d) Ensuring that potential complications during and after the procedure are avoided or minimized, that animals are monitored for complications, and that any complications that do occur are effectively managed. Potential complications include hypothermia, dehydration, blood loss, tissue trauma, metabolic disturbances, poor tissue perfusion and cardiovascular or respiratory failure (or both), infection, delayed wound healing and impaired function.

e) Ensuring that pain management that is appropriate for the species and the procedure is effective, and includes effective anaesthesia as well as avoiding and minimizing postoperative pain, suffering, distress and lasting harm.

f) Ensuring that, for non-recovery surgery, the animal remains unconscious throughout the procedure and death is confirmed at the end of the procedure.

g) Ensuring that animals that undergo more than one surgical procedure have recovered to good general health before any subsequent procedure is performed, unless otherwise approved by the AEC.

6.4.8 Post procedure care

6.4.8.1 After any procedure:

a) The animals shall be monitored and assessed with sufficient frequency to ensure that both predicted and unforeseen consequences are detected early (see 6.3.1 and 6.3.3.3.1 to 6.3.3.3.2 (inclusive)). If an animal has undergone a surgical procedure, surgical wounds shall be inspected regularly for evidence of infection, dehiscence of wounds and progress of healing.

b) Prompt action shall be taken so that predicted and unforeseen consequences, including pain, suffering, distress and lasting harm, are addressed rapidly and effectively (see 6.3.3.3.4 to 6.3.3.3.5 (inclusive)).

c) Appropriate care and supportive treatment that will support and safeguard animal well-being shall be provided, including nursing of the animal, physiotherapy, pharmacological management of pain, suffering, distress and lasting harm, provision of fluid and nutritional support, and prevention or control of infection. and

d) Appropriate records shall be maintained and made accessible to all people involved in the post procedural care of the animal (see 5.5.3.5.1 to 5.5.3.5.4 (inclusive), 5.6.3.5.1 and 6.3.3.3.3).

6.4.8.2 If an animal shall be housed in isolation or separated from their group after a procedure, the duration of such housing conditions should be minimized. Where appropriate for the species and the individual animal, and as far as possible, the animal should be able to see, hear and smell animals of the same species unless such contact will interfere with data collection and interpretation (see 6.3.3.1.8).

6.4.8.3 If an animal is to be isolated or restrained for a prolonged period after a procedure, the animal should be adequately conditioned to the housing or restraint conditions before the procedure is undertaken (see 6.3.3.1.7 and 10.6.3.6.3).

6.4.8.4 Animals that have undergone surgery for transplantation of organs or tissues shall be managed to avoid or minimize adverse impacts from potential rejection of the transplant and the effects of immunosuppression.

6.4.9 Projects involving the fetus or embryo

6.4.9.1 Where a project involves the fetus or embryo, the requirements for anaesthesia and analgesia of the fetus or embryo shall be taken into account (see 6.4.6.1 to 6.4.6.8 (inclusive)).

6.4.9.2 If a procedure conducted on a fetus or embryo would compromise the ability of the animal to survive after birth or causes untreatable pain, suffering and distress, the animal (neonate/fetus/embryo) shall be killed humanely before or immediately after birth.

6.4.10 Induction of tumours

For animals in studies that involve the induction of tumours, methods used and endpoints chosen shall ensure that valid results are obtained with minimal harm, including pain, suffering and distress, to the animal. Animal well-being shall be supported and safeguarded by

a) Considering potential adverse impacts associated with the development and biology of the tumour (including growth rate, invasiveness, potential for ulceration, development of metastases and cachectic effects), effects of therapeutic agents, side effects of immunotherapy including irradiation, and consequences of surgery involved in transplantation of tumours.

b) Choosing an appropriate implantation site or method of induction of the tumour that causes the least harm, including pain, suffering and distress, to the animal. The footpad, tail, brain or eye shall not be used unless there is no valid alternative.

c) Monitoring the growth or impact of the tumour and efficacy of therapy, and using early experimental endpoints, to obtain valid results as early as possible. Death from the tumour shall not be an endpoint.

d) Establishing and implementing early intervention points and humane endpoints (see 6.3.3.4.1 to 6.3.3.5.1 (inclusive)).

e) Wherever possible, using techniques that facilitate measurement of tumour growth and determination of early endpoints.

f) Monitoring and assessing animals for signs of pain, suffering, distress and lasting harm, including changes in body condition and body weight; ulceration; adverse effects of procedures used for induction of the tumour; signs of growth, invasion and metastases of the tumour; and toxic effects of therapeutic agents.

6.4.11 Creation and breeding of new animal lines where the impact on animal well-being is unknown or uncertain

6.4.11.1 This clause shall be read in conjunction with 5.5.3.4.4.1 to 5.5.3.4.4.2 (inclusive) and the NHMRC *Guidelines for the generation, breeding, care and use of genetically modified and cloned animals for scientific purposes* (see 2.2).

6.4.11.2 When creating and breeding new animal lines where the impact on animal well-being is unknown or uncertain, the well-being of the animals shall be supported and safeguarded by

a) considering the nature and extent of potential impact on animal well-being due to

   1) genetic modification and the difficulty in predicting the potential impact,

   2) the procedures used to create a new animal line

b) using methods for the generation, monitoring and phenotypic description of a new animal line that are in accordance with current best practice,

c) using the least invasive method for genotyping, and identifying the animal appropriately to allow the genotype result to be matched to the animal, and

d) assessing the impact of genetic modification on the well-being and genetic stability of newly created genetically modified animals and their offspring across a number of generations (see 5.5.3.4.4.1).

6.4.12 Modification of behaviour and neurological function

6.4.12.1 Positive reinforcement should be used to motivate an animal to modify their behaviour or perform specific tasks.

6.4.12.2 Prolonged deprivation of water, food, social interaction or sensory stimuli shall not be used to induce an animal to modify their behaviour. All deprivation need to be scientifically justified and approved by AEC.

6.4.12.3 If some form of biological stress is essential for the aims and purpose of the project, the duration and severity of the impact on the well-being of the animal shall be as mild as possible.

6.4.12.4 Painful or noxious stimuli should be avoided. If their use is justified, the level and duration of the stimulus shall be minimized, and provision shall be made for the animal to be able to escape the stimulus unless otherwise justifiable.

6.4.12.5 Projects involving the withholding or restriction of food or water shall be designed so that the animal experiences no continuing detrimental effect. Changes in fluid balance, body weight or body conditioned score shall be monitored, recorded and maintained within the limits approved by the AEC.

6.4.12.6 When a study involves neurological impairment that produces loss of function in the animal (for example, impaired movement of the limbs or trunk; loss of sensibility to touch, sound, temperature or pain, or awareness of surroundings; or impairment of appetite or thirst), the special needs of the animal because of that loss of function shall be met. Such animals should be provided with special care, caging and other facilities, as required.

6.4.13 Immunomodulation and production of antibodies

6.4.13.1 When agents or treatments are used to suppress the immune system, (for example, irradiation)

a) procedures to minimize the risk of infection shall be followed, and

b) animals shall be appropriately monitored so that potential side effects are promptly identified and effectively managed.

6.4.13.2 When adjuvants are used to produce antibodies, the adverse impacts on animal well-being should be minimized by

a) using an adjuvant that provides an adequate antibody titre while causing the least adverse impact on the well-being of the animal,

b) using a ratio of adjuvant to antigen that reduces the probability of adverse reactions,

c) choosing the volume, site and frequency of injection of adjuvant that together optimise the antibody response and minimize the risk of complications, and

d) choosing a method and frequency of blood sampling that minimize the potential for harm, including pain, suffering and distress.

6.4.14 Toxicological studies

6.4.14.1 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 competent persons. 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.

6.4.14.2 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, suffering, distress and lasting harm.

6.4.14.3 The researchers shall not allow scientific studies to proceed to the point of painful, distressful or lingering death of animals.

6.4.14.4 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.

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

6.4.15.1 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 (see 5.2.3.1.2(i)).

6.4.15.2 The endpoint of scientific studies or teaching activities that involve hazardous agents shall conform to the requirements for toxicological studies (see 6.4.14.1 to 6.4.14.4 (inclusive)).

6.4.16 Animal welfare and animal health research

When studying ways of improving the health or welfare of animals, the 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 6.4.14.1 to 6.4.14.4 (inclusive)).

6.4.17 Study manipulation of animals' genetic material

6.4.17.1 Work that involves the introduction of foreign DNA into mammalian cells or whole animals shall be conducted in accordance with guidelines issued by the relevant national legislation (see foreword), and the relevant biohazards committee of the institution.

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

6.4.17.3 The manipulation of the genetic material of animals has the potential to affect the welfare of the animals and their offspring adversely. The researchers shall inform the AEC of the known potential adverse effects on the well-being of the animals.

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

6.4.18 Lesions of the central nervous system

6.4.18.1 Studies of anatomical, chemical or other 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.

6.4.18.2 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.

6.4.19 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, the researchers shall

a) ensure that these stimuli limit pain at all times to levels that do not cause severe pain or distress (or both),

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.

6.4.20 Provisions for animals at the conclusion of their use

6.4.20.1 Opportunities to rehome animals should be considered wherever possible, especially when the impact of the project or activity on the well-being of the animal has been minimal and their physiological condition and behavioural attributes indicate that they can be introduced to a new environment with minimal, transient impact on their well-being.

6.4.20.2 An animal shall not be released to a person at the conclusion of their use unless

a) The AEC has approved such release.

b) Safeguards are in place and approved by the AEC to ensure the on-going well-being of the animal. In the case of primary and secondary level students, safeguards shall include a written commitment from a parent or guardian for the provision of adequate, on-going and responsible care of the animal, and demonstrating an awareness of relevant legislative requirements as specified in the relevant national legislation (see foreword), regarding the animal being rehomed.

c) Transport of animals between sites is in accordance with 10.2.

6.4.20.3 The return of animals to normal husbandry conditions and the release of wildlife to their natural habitat shall be in accordance with current best practice.

6.4.20.4 The method and procedures used for killing an animal shall comply with 6.4.21.1.

6.4.20.5 The number of animals used may be reduced by the appropriate reuse of individual animals. When considering approval for the reuse of animals, the AEC shall take into account

a) the pain, suffering, distress and lasting harm, and any potential long-term or cumulative effects, caused by previous activities and conditions,

b) the time allowed for recovery of the animals between activities,

c) whether an animal has fully recovered from the previous activities,

d) the pain, suffering, distress and lasting harm likely to be caused by the next and subsequent activities, and

d) the total time over which an animal will be used.

6.4.20.6 Investigators should ensure that, if practicable, tissue samples from the animals that have died or been humanely killed are provided or made available to other investigators for their work, or deposited in a tissue bank for subsequent distribution.

6.4.21 Humane killing

6.4.21.1 The method and procedures used for killing an animal shall be humane and

a) avoid pain or distress and produce rapid loss of consciousness until death occurs,

b) be compatible with the purpose and aims of the project or activity,

c) be appropriate to the species, age, developmental stage and health of the animal,

d) require minimum restraint of the animal use of sedation should be considered,

e) be reliable, reproducible and irreversible,

f) ensure that animals are killed in a quiet, clean environment away from other animals, and

g) ensure that death is established before disposal of the carcass, foetuses, embryos and fertilized eggs (see table 1).

6.4.21.2 Dependent offspring of animals to be killed shall be cared for or humanely killed.

Table 1 — Methods of killing animals

1	2	3	4	5	6	7	8	9	10
Animals	**Anaesthetic overdose**	**Inert gases(Ar, N_2)**	**Captive bolt, followed by exsanguination**	**Carbon dioxide**	**Cervical dislocation**	**Concussion/ percussive blow to the head**	**Decapitation**	**Electrical stunning, followed by exsanguination**	**Shooting with a free bullet with appropriate rifles, guns and ammunition**
Fish	(1)					(2)		(3)	
Amphibians	(1)					(2)		(3)	
Reptiles	(1)		(4)			(2)	(5)		(6)
Birds	(1)	(7)		(7)	(8)		(9)	(3)	(6)
Rodents	(1)	(10)(11)		(10)(11)	(12)		(2)(3)		
Rabbits	(1)		(3)		(13)			(3)	
Dogs, cats	(1)								(6)
Large mammals	(1)	(11)	(3)					(3)	(6)
Non-human primates	(1)								(6)
All euthanasia methods shall be performed by highly skilled and competent person. Method shall not be used, as method is not applicable:									

Table 1 — Methods of killing animals *(concluded)*

NOTE The most recent scientific literature should be consulted to ensure these methods are still ethically acceptable at the time of the study.

1) Method shall, where appropriate, be used with prior sedation.

2) Method only to be used if other methods are not possible.

3) Specialized equipment required.

4) Method only to be used on large reptiles.

5) Decapitation shall be followed with destruction of the brain.

6) Method only to be used in field conditions by experienced marksmen when other methods are not possible.

7) Method always to be used in combination with an inhaled anaesthetic or inert gas.

8) Birds over 250 g shall be sedated. Method only to be used for birds under 1 kg.

9) Method only to be used for birds under 250 g.

10) Inhalant gases not to be used for foetal and neonate rodents.

11) Animal shall be anaesthetised prior to CO_2 or inert gases being administered.

12) Rodents over 150 g shall be sedated. Method only to be used for rodents under 500 g.

13) Rabbits over 150 g shall be sedated. Method only to be used for rabbits under 1 kg.

7 The care and use of animals for the achievement of educational outcomes in science

7.1 General

7.1.1 This clauses covers the care and use of animals in teaching activities where the 'scientific purpose' is to impart or demonstrate knowledge or techniques to achieve an educational outcome in science, as specified in the relevant curriculum or competency requirement. All parts of the standard apply to teaching activities. This clauses provides additional guidance on the responsibilities outlined in other clauses when animals are used for teaching activities. It shall be applied in addition to other parts of the standard, particularly clauses 5 and 6.

7.1.2 It is expected that animals used in teaching activities will receive a high standard of care, that their well-being will be supported and safeguarded in accordance with the governing principles outlined in clause 4, and that their use will occur only in accordance with approval from the AEC.

7.2 Governing principles

Each person involved in the care and use of animals for scientific purposes shall consider the governing principles in clause 4 when applying this standard to their specific circumstance; in particular:

a) respect for animals shall underpin all decisions and actions involving the care and use of animals for scientific purposes (see 4.2.1).

b) the obligation to respect animals, and the responsibilities associated with this obligation, apply throughout the animal's lifetime, including acquisition, transport, breeding, housing, husbandry, use of the animal in a project, and provisions for the animal on completion of their use (see 4.2.4).

c) people involved in any aspect of the care and use of animals for scientific purposes shall be aware of and accept their responsibilities, and act in accordance with this standard (see 4.7.4.1).

d) all activities, including projects that involve the care and use of animals for scientific purposes shall

1) be subject to ethical review, approval and monitoring by the AEC,

2) commence only after approval has been granted by the AEC,

3) be conducted in accordance with the AEC approval, and

4) cease immediately if approval from the AEC is suspended or withdrawn or period of said approval ends (see 4.7.4.2).

7.3 Responsibilities

7.3.1 Institutions

7.3.1.1 General

7.3.1.1.1 The responsibilities of institutions involved in the care and use of animals for teaching activities are as outlined in 5.2.

7.3.1.1.2 The responsibilities of institutions regarding the governance of the AEC overseeing the care and use of animals in teaching activities are as outlined in 5.3.

7.3.1.1.3 The institutions shall ensure that animals are used for teaching only when their use is essential to achieve an educational outcome in science, as specified in the relevant curriculum or competency requirements, and suitable alternatives to replace the use of animals to achieve the educational outcome are not available (see 4.3.1).

7.3.1.1.4 The institutions shall identify the person with ultimate responsibility for the care and use of animals in teaching activities. This person shall

a) ensure that all people involved in the care of animals understand and accept their role and responsibilities,

b) ensure that procedures and resources are in place so that all people involved in the care and use of animals can meet their responsibilities, and

c) be competent with respect to the well-being of animals under their care.

NOTE This person does not relieve the individual responsibility of the teacher who is involved in the care and use of animals in teaching activities.

7.3.1.2 Primary and secondary educational institutions (all schools including agricultural schools)

7.3.1.2.1 Institutions involved in the care and use of animals in teaching activities in the primary and secondary sectors shall ensure that they have access to the AEC. This may be a regional or external AEC (see 5.2.3.2.2).

7.3.1.2.2 The institutions shall ensure that the following activities using animals are not demonstrated to, or carried out by, primary or secondary level students, as these procedures are considered emotionally distressing to people and are potentially distressing to the animals used in the experiment and also could potentially result in the unnecessary use of animals for experimentation. If the following procedures have to be undertaken, it should be based on a full research protocol approval by the AEC and make use of fully qualified, registered or authorized staff working within an appropriate facility:

a) animal breeding that does not achieve an educational outcome in science and fails to provide for the lifetime welfare of animals (and their offspring, if relevant);

b) surgical, invasive and other harmful procedures, other than routine husbandry procedures;

c) induction of an infectious disease or illness;

d) production of nutritional deficiency;

e) exposure to conditions that would cause an animal to experience pain and distress;

f) administration of drugs or chemicals unless for therapeutic or diagnostic purposes; and

g) administration of toxins, ionising radiation or biohazards.

7.3.1.2.3 Institutions shall ensure that humane killing of animals is not demonstrated to, or carried out by, primary or secondary level students unless it is required

a) to achieve an educational outcome in science as specified in the relevant formally approved curriculum or competency requirement, or

b) as part of veterinary clinical management of an animal, under the direction of a veterinarian.

7.3.2 The AEC

Responsibilities of the AEC overseeing the care and use of animals in teaching activities (see 5.4).

7.3.3 Teachers as investigators and animal carers

7.3.3.1 When teachers use animals for teaching activities, the teacher has the responsibilities of the investigator under 5.5.

7.3.3.2 When teachers are responsible for the care of animals that are used for teaching activities, including during their acquisition, transport, breeding, housing and husbandry, the teacher has the responsibilities of an animal carer under 5.6.

7.3.3.3 Teachers have personal responsibility for all matters that relate to the well-being of animals that they use, including their housing, husbandry and care. This responsibility extends throughout the period of use approved by the AEC until provisions are made for the animal at the conclusion of their use (consistent with 5.5.3.1.1).

7.3.3.4 Teachers shall ensure that students have the opportunity to discuss the ethical, moral and social issues, and legal responsibilities, involved in the care and use of animals for scientific purposes, at a level appropriate to their learning ability and comprehension, and before the use of animals commences. This shall include the philosophical principles (see 5.10.3.1) and the four Rs (see 5.10.3.2) and national standards and legislation (see 5.10.2.3).

7.3.3.5 Teachers shall ensure that the students are adequately supervised by a person who is competent for the procedure being performed, and that the level of supervision of students takes into account the competency and responsibilities of each student as well as the number of students and number of animals involved.

7.3.3.6 Teachers shall ensure that animals are not released to students, or any other person, for temporary care, or at the completion of the use of the animal (see 6.4.20.1 to 6.4.20.2 (inclusive)), unless

a) The AEC has approved such release.

b) Safeguards are in place and approved by the AEC to ensure the on-going well-being of the animal. In the case of primary and secondary level students, safeguards shall include a written commitment from a parent or guardian for the provision of adequate, on-going and responsible care of the animal, and demonstrating awareness of relevant legislative requirements regarding the animal being rehomed or under their care.

c) Transport of animals between sites is in accordance with 10.2.

d) Health and safety of the humans and in contact animals should be appropriately considered.

e) If this should be in line with the relevant regulatory authorities (see foreword). In South Africa it may be required that the re-homing of animals is approved by, but not limited to, the relevant regulatory authorities (see foreword).

7.3.4 Obtaining approval from an AEC

7.3.4.1 Teachers, and the person with ultimate responsibility for a teaching activity, shall follow institutional and AEC procedures when submitting an application to the AEC (see 5.8) and provide information in the application form as outlined in 5.8.3.2. The AEC may be a regional, provincial or national AEC (see 7.3.1.2.1).

7.3.4.2 The AEC approval may be sought to repeat a particular teaching activity that may involve different students, times, locations or animals (see 5.5.3.6).

7.3.4.3 The AEC approval is not required for the training and application of agricultural extension work practices, or the training of students in veterinary science, veterinary nursing or animal technology to achieve competency-based outcomes in routine procedures if all of the following apply:

a) the animals are at their home property (with the exception of animals owned by the facility undertaking the said training, or animals owned by a premise registered with the regulatory body (see foreword), in which case AEC approval is required),

b) the procedures would normally occur as part of routine management or veterinary clinical management of the animal,

c) the animals are not subjected to anything additional to routine management or veterinary clinical management of the animal, and

d) under the direct supervision of a teacher who is competent to carry out the procedure.

8 Complaints and non-compliance

8.1 General

8.1.1 The institutions may receive complaints about the care and use of animals for scientific purposes. Complaints may be raised by any person or group, including investigators, animal caretakers, AECs, the AEC members, students, employees of the institution and members of the public. Complaints may relate to the activities of any party or person involved in the care and use of animals, including the investigators, the animal caretakers, the AEC and governance officials. Institutions may also become aware of activities relating to the care and use of animals for scientific purposes that are not being conducted in accordance with this standard.

8.1.2 This clause outlines the responsibilities of the institutions and the AECs for addressing complaints and non-compliance relating to the care and use of animals for scientific purposes.

8.2 Governing principles

Each person involved in the care and use of animals for scientific purposes shall consider the governing principles in clause 4 when applying the standard to their specific circumstance; in particular:

a) the Institutions, AECs, and people involved in any aspect of the care and use of animals for scientific purposes shall be aware of and accept their responsibilities (see 4.7.4), and act in accordance with this standard (see 4.7.4.1).

b) all activities, including projects that involve the care and use of animals for scientific purposes shall

   1) be subject to ethical review, approval and monitoring by the AEC,

   2) commence only after approval has been granted by the AEC,

   3) be conducted in accordance with the AEC approval, and

   4) cease if approval from the AEC is suspended or withdrawn or period of said approval ends (see 4.7.4.2).

8.3 Responsibilities of institutions on complaints and non-compliance

8.3.1 General

8.3.1.1 The institutions shall have procedures for addressing complaints and non-compliance relating to the care and use of animals for scientific purposes, including

a) Complaints concerning the care and use of animals by the institution, including conscientious objection in the case of teaching activities.

b) Complaints concerning the AEC process of review of an application or report, including resolution of disagreements between AEC members, between the AEC and the investigators, and between the AEC and the institution.

c) Complaints concerning the process for independent external review.

d) Non-compliance with this standard by any party or person involved in the care and use of animals including investigators, animal carers, the AEC, governance officials, and external parties subject to agreements described in 5.7.3.3 and 5.7.4.1.3. Non-compliance may also involve breaches of the relevant national legislation (see foreword), and the institutions should have procedures for advising the relevant regulatory authorities (see foreword) (see 8.3.3.2.2).

8.3.1.2 The institutional procedures shall

a) give priority consideration to the well-being of the animals, and ensure that activities with the potential to adversely affect animal well-being cease immediately,

b) clearly define the mechanisms for receiving, investigating and addressing complaints,

c) clearly define the mechanisms for addressing non-compliance with this standard,

d) clearly define the responsibilities of all parties,

e) ensure fair, prompt, timely, effective, confidential processes that accord with procedural fairness, the principles of natural justice and protection of whistle-blowers,

f) identify and ensure appropriate reporting to the institution, the AEC, the state or the territory government authorities, and any other relevant bodies, and

g) be made available to all relevant people.

8.3.1.3 For projects involving more than one institution or AEC (or both) (see 5.7.4.1.1 to 5.7.4.1.4 (inclusive)), procedures should include mechanisms for reporting between the relevant institutions and the AECs on complaints and non-compliance.

8.3.2 Receiving, investigating and addressing complaints

8.3.2.1 Complaints concerning the care and use of animals

8.3.2.1.1 The institutions shall ensure that

a) where complaints relate to activities that have the potential to adversely affect animal well-being, the activities cease immediately,

b) where complaints relate to activities that would normally require the AEC approval, the complaints are referred to the AEC to investigate whether such activities are conducted in accordance with the AEC approval,

c) where complaints raise the possibility of ″research misconduct″, as described in standard for the responsible conduct of research, the complaint is handled in accordance with procedures specified in that document, and

d) where complaints allege misconduct that falls outside the range of ″research misconduct″, as described in this standard for the responsible conduct of research, the complaint is handled in accordance with institutional processes for dealing with other forms of misconduct.

8.3.2.1.2 Following the AEC's investigation of complaints referred to it by the institution, the AEC

a) Shall ensure that, where activities are conducted in accordance with the AEC approval, the activities are reviewed in consultation with all relevant people to ensure that the reason for the complaint is addressed. The AEC may decide that modification to a project or activity is required, or an approval for a project or activity is suspended or withdrawn or period of said approval ends.

b) Should ensure that, where activities are not conducted in accordance with the AEC approval, the matter is referred back to the institution for action.

8.3.2.2 Complaints concerning the animal ethics committee process

Where complaints concerning the AEC process of review of an application or report cannot be resolved by communication between the complainant and the AEC that is the subject of the complaint, the institution should ensure that the complainant has access to a person or agency external to the AEC for review of the process followed by the AEC. This person or agency may be within the institution. Following this review, the AEC may need to review its process in reaching its decision regarding the application or report, and re-evaluate its decision in light of the reviewed process. The ultimate decision regarding the ethical acceptability of an activity lies with the AEC and shall not be overridden.

8.3.2.3 Complaints concerning the process for independent external review

Institutions shall ensure that the process for conducting an independent external review, developed in consultation with the review panel, includes an appeals process that relates to the process for the review (see 9.3.2.2).

8.3.2.4 Referral to a person or agency external to the institution

The institutions should identify a person or agency external to the institution to whom a person can take a complaint that has not been resolved by the processes referred to in 8.3.1.1 to 8.3.1.3 (inclusive).

8.3.3 Addressing non-compliance

8.3.3.1 General

8.3.3.1.1 The Institutions shall have procedures for addressing non-compliance with this standard, so that behaviours that create and support compliance are encouraged, and behaviours that compromise compliance are not tolerated.

8.3.3.1.2 The institution shall maintain records of breaches of this standard.

8.3.3.2 Advising regulatory authorities

8.3.3.2.1 Any person can report alleged breaches of legislation to the relevant regulatory authorities (see foreword).

8.3.3.2.2 The institution should advise the relevant regulatory authorities (see foreword) of alleged breaches of legislation that had a significant impact on animal well-being.

9 Independent external review of the operation of institutions

9.1 General

Independent external review assists institutions to assess whether the procedures they have established meet the goals set out in the standard, and provides assurance that the institution, through its animal ethics committee (AEC), is delivering effective oversight of the care and use of the animals in its charge. The process should be educational and provide an opportunity for self-assessment so that members of the AEC and those at the institution who have responsibilities for animal care and use are involved in achieving the desired outcomes.

9.2 Governing principles

Each person involved in the care and use of animals for scientific purposes shall consider the governing principles in clause 4 when applying this standard to their specific circumstance; in particular

a) the institutions, the AECs, and the people involved in any aspect of the care and use of animals for scientific purposes shall be aware of and accept their responsibilities (see clause 5), and act in accordance with the standard (see 4.7.4.1),

b) all activities, including projects, that involve the care and use of animals for scientific purposes shall

   1) be subject to ethical review, approval and monitoring by the AEC,

   2) commence only after approval has been granted by the AEC,

   3) be conducted in accordance with the AEC approval, and

   4) cease if approval from the AEC is suspended or withdrawn or period of said approval ends (see 4.7.4.2).

9.3 Responsibilities

9.3.1 Institutions

9.3.1.1 Institutions shall

a) ensure that an independent external review is conducted at least every four years to assess the institution's compliance with this standard, and

b) ensure the continued suitability, adequacy and effectiveness of its procedures to meet its responsibilities under this standard (see 4.4.2(a)).

9.3.1.2 Institutions shall

a) Make arrangements for the review to be conducted by external people who are independent of the institution and the activities conducted on behalf of the institution, and who have appropriate or experience relevant to the activities of the institution (or all of these).

b) Establish procedures so that members of the review panel declare their interests, and any conflicts of interest are managed.

c) Ensure that members of the review panel are advised of requirements for confidentiality.

d) Provide the review panel with the necessary authority and resources to conduct the independent review of the activities of the institution. This will include access to people, information, records and premises, and provision of reasonable assistance.

e) Ensure that the findings and recommendations of the review are made widely known within the institution.

f) Ensure that timely actions are taken to address the recommendations of the review.

g) Consider publishing a summary of the external review report (for example, as part of an institutional annual report or website) and making the summary report available to the relevant regulatory authority and funding bodies of the institution (see 5.2.3.7.2).

9.3.1.3 Reviews carried out under the administration of state or territory legislation may satisfy the requirement for an independent external review.

9.3.2 Review panel

9.3.2.1 Members of the review panel shall

a) declare their interests before their appointment to the review panel, and

b) adhere to confidentiality requirements regarding the review.

9.3.2.2 The review panel shall

a) Develop a process for the manner in which the review should be conducted, in consultation with the institution. This process should accord with the principles of natural justice and include an avenue for the process and the outcomes to be appealed.

b) Document the findings and recommendations from the review and provide a report to the governing body of the institution.

9.4 Scope and outcomes of the independent external review

9.4.1 Areas covered by the independent external review should include

a) the conduct of all people involved in the care and use of animals for scientific purposes on behalf of the institution, including the AEC, institutional officers and administrators, investigators and animal carers,

b) the adequacy of the institutional programme to ensure that the care and use of animals for scientific purposes is conducted in compliance with the standard; is subject to ethical review, approval and monitoring by an AEC; and is conducted in accordance with the AEC approval,

c) the adequacy of institutional support, resources and educational programmes for the AEC and its members, and for people involved in any aspect of the care and use of animals for scientific purposes, to ensure that they can meet their responsibilities under this standard,

d) whether the AEC is operating effectively in accordance with this standard,

e) the effectiveness of institutional strategies to promote and monitor the implementation of the governing principles,

f) whether there is effective monitoring of the well-being of animals,

g) whether facilities used to house animals are managed to support and safeguard animal well-being, and

h) if applicable, an assessment of the report from the previous external review and actions taken in response to recommendations in that report.

9.4.2 The review panel should provide recommendations that

a) identify areas of non-compliance,

b) support strategies for short-term and long-term continual improvement, and

c) give recognition to behaviours and actions by individuals and teams that support compliance.

10 Requirements for facilities for the care and housing of animals

10.1 General

This clause covers the requirements for acquisition, admission to a facility and transportation of animals within South African borders, internationally and locally within a facility. Physical requirements for the care and housing of animals used for scientific purposes be that teaching activities, breeding programmes or research projects, are described. Such requirements include layout and design of physical facility, environmental controls (temperature, ventilation, lighting, noise, vibrations etc.) and care of the animals (health, housing, enrichment, feeding, water, handling, breeding etc.). This clause also includes species-specific minimum housing requirements.

10.2 Transport of animals

10.2.1 Local transport of livestock should comply with the relevant national standards, for example, SANS 1488 and, where applicable, the requirements for the transport of wild herbivores in SANS 10331, and any relevant permits as required by the relevant regulatory authority (see foreword).

10.2.2 Methods and arrangements for the transport of animals shall support and safeguard the well-being of the animals before, during and after their transport, and take into account the health, temperament, age, sex and previous experiences of the animals; the number of animals travelling together and their social relationships; the period without food or water; the duration and mode of transport; environmental conditions (particularly extremes of temperature); and the care given during the journey.

10.2.3 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.

10.2.4 Food and water shall be provided depending on environmental condition and temperatures. As a general guide water should be provided every 2 h to 6 h and ungulates and pachyderms shall be provided with roughage during transport. In general most species should be rested after a 12 h period.

10.2.5 Transport arrangement depends on species and demeanour. Temperature in transport container should ideally be monitored to ensure preferred temperature range for species.

10.2.6 The suppliers, transporters and recipients of animals shall ensure that there are satisfactory delivery procedures and facilities and that animals are received by a person responsible for animal care. Responsibility should be outlined in form of a written agreement between the parties involved, including contingency plans during emergencies.

10.2.7 Transport shall be documented in the facilities SOPs.

10.2.8 People responsible for monitoring animals during transport shall be able to recognise and respond to animal needs during transport, loading and offloading.

10.3 Acquisition of animals from facilities within the Republic of South Africa

10.3.1 Animals shall be obtained from breeding and supply facilities that maintain conditions and uphold standards consistent with this standard, the relevant national legislation (see foreword) and relevant industry standards.

10.3.2 Animals which are for use in scientific studies and teaching activities, and of the species listed in table 2, 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 2 — Animals for use in scientific studies and teaching activities

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

10.3.3 The institutions shall undertake to extend the provisions of 10.3.1 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.

10.3.4 Stray and feral animals of domestic species shall not be used in procedures.

10.3.5 The AEC may only grant exemptions from 10.3.4 subject to the following conditions:

a) there is an essential need for studies concerning the health and welfare of the animals or serious threats to the environment or to human or animal health; and

b) there is scientific justification to the effect that the purpose of the procedure can be achieved only by the use of a stray or a feral animal.

10.3.6 Permits to trap, hold, transport and import or export indigenous species between provinces in the South Africa shall also be obtained from the various nature conservation authorities and relevant regulatory authority (see foreword) or any other relevant national legislation (see foreword).

10.4 Acquisition of animals from other countries

10.4.1 Animal imports will only be allowed if the importer is in possession of the necessary import permits and veterinary health certificates from the respective country of origin.

10.4.2 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 or industry standard (or both). 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.

10.5 Admission, acclimatization and conditioning of new animals into holding facilities

10.5.1 When new animals are admitted to breeding and holding facilities, their well-being shall be supported and safeguarded by

a) ensuring that the health and well-being of the animals is assessed by a person registered or authorized by the relevant national council (see foreword) before their admission, and quarantine and preventive or other health treatment is provided, if appropriate,

b) ensuring that appropriate housing is available and that animals are transferred to this housing without unnecessary delay,

c) assessing the suitability of the animals for their intended scientific purpose, and

d) providing a relevant acclimatization period approved by the AEC refer to the study guidelines relevant to the type of study (for example, Organization for Economic Cooperation and Development (OECD) guidelines) or the annexes for the specific species given in this standard.

10.5.2 The animals that do not adapt satisfactorily after acclimatization or conditioning (or both) should not be used, and prompt provisions should be made for such animals, as appropriate.

10.6 General requirements for physical facilities

10.6.1 The physical facilities

10.6.1.1 Functions and general design

10.6.1.1.1 All facilities shall be constructed so as to provide an environment which takes into account the physiological and ethological needs of the species kept in them. Facilities shall also be designed and managed to prevent access by unauthorized persons or products and the ingress or escape of animals.

10.6.1.1.2 Establishments shall have an active maintenance programme to prevent and remedy any defect in buildings or equipment.

10.6.1.1.3 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.

10.6.1.1.4 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. Confinement is associated with greater risk to disease, injury and bullying by conspecifics. Vigilant health monitoring will be required.

10.6.1.2 Holding rooms

10.6.1.2.1 Establishments shall have a regular and efficient cleaning schedule for the rooms and shall maintain satisfactory hygienic standards. Cleaning agents should be non-toxic to the animals and of a nature that will not interfere with the study outcome.

10.6.1.2.2 Walls and floors shall be surfaced with a material resistant to the heavy wear and tear caused by the animals and the cleaning process. The material shall not be detrimental to the health of the animals and shall be such that the animals cannot hurt themselves. Additional protection shall be given to any equipment or fixtures so that they are not damaged by the animals nor do they cause injury to the animals themselves.

10.6.1.2.3 Species that are incompatible, for example, predator and prey, or animals requiring different environmental conditions, shall not be housed in the same room nor in such proximity as to cause stress to the prey species.

10.6.1.3 General and special purpose procedure area i.e. room/camp/pasture

10.6.1.3.1 Establishments shall, where appropriate, have available laboratory facilities for the carrying out of simple diagnostic tests, post-mortem examinations, or the collection of specimens that are to be subjected to more extensive laboratory investigations elsewhere. General and special purpose procedure areas shall be available for situations where it is undesirable to carry out the procedures or observations in the holding rooms.

10.6.1.3.2 Facilities shall be provided to enable newly-acquired animals to be isolated until their health status can be determined and the potential health risk to established animals assessed and minimized.

10.6.1.3.3 There shall be housing for the separate housing of sick or injured animals.

10.6.1.4 Utility areas

10.6.1.4.1 Store-rooms shall be designed, used and maintained to safeguard the quality of food and bedding. These rooms shall be vermin and insect-proof, as far as possible. Other materials, which may be contaminated or present a hazard to animals or staff, shall be stored separately.

10.6.1.4.2 The cleaning and washing areas shall be large enough to accommodate the installations necessary to decontaminate and clean used equipment. The cleaning process shall be arranged so as to separate the flow of clean and dirty equipment to prevent the contamination of newly-cleaned equipment.

10.6.1.4.3 Establishments shall provide for the hygienic storage and safe disposal of carcasses and animal waste.

10.6.1.4.4 Where surgical procedures under aseptic conditions are required, there shall be provision for one or more than one suitably equipped room, and facilities provided for postoperative recovery.

10.6.1.4.5 There shall be a separate storage area for flammables and controlled substances that is lockable and secure with access control, and that complies with the relevant national legislation (see foreword).

10.6.2 The environment and control thereof

10.6.2.1 Ventilation and temperature

10.6.2.1.1 Insulation, heating and ventilation of the holding room shall ensure that the air circulation, dust levels, and gas concentrations are kept within limits that are not harmful to the animals housed.

10.6.2.1.2 Temperature and relative humidity in the holding rooms shall be adapted to the species and age groups housed. The temperature shall be measured and logged on a daily basis.

10.6.2.1.3 The animals shall not be restricted to outdoor areas under climatic conditions which may cause them stress or be inappropriate for the species.

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

10.6.2.2 Lighting

10.6.2.2.1 Where natural light does not provide an appropriate light or dark cycle, controlled lighting shall be provided to satisfy the biological requirements of the animals and to provide a satisfactory working environment.

10.6.2.2.2 Illumination shall satisfy the needs for the performance of husbandry procedures and inspection of the animals.

10.6.2.2.3 Regular photoperiods and intensity of light adapted to the species shall be provided.

10.6.2.2.4 When keeping albino animals, the lighting shall be adjusted to take into account their sensitivity to light.

10.6.2.3 Noise

10.6.2.3.1 Noise levels including ultrasound, shall not adversely affect animal welfare.

10.6.2.3.2 Establishments shall have appropriate alarm systems that sound outside the sensitive hearing range of the animals, where this does not conflict with their audibility to human beings.

10.6.2.3.3 Holding rooms shall where appropriate be provided with noise insulation and absorption materials.

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

NOTE Separation of human and animal areas will minimize disturbances.

10.6.2.4 Alarm systems

10.6.2.4.1 Establishments relying on electrical or mechanical equipment for environmental control and protection, shall have a stand-by system to maintain essential services and emergency lighting systems as well as to ensure that alarm systems themselves do not fail to operate. There shall be adequate contingency plans to cover emergencies such as the breakdown of lighting, heating or cooling, and fire or flood.

10.6.2.4.2 Heating and ventilation systems shall be equipped with monitoring devices and alarms.

10.6.2.4.3 Clear instructions on emergency procedures shall be prominently displayed.

10.6.3 Care of animals

10.6.3.1 Health

10.6.3.1.1 Establishments shall have a strategy in place to ensure that a health status of the animals is maintained that safeguards animal welfare and meets scientific requirements. This strategy shall include regular health monitoring, a microbiological surveillance programme and plans for dealing with health breakdowns or emergencies (for example, fire) and shall define health parameters and procedures for the introduction of new animals.

10.6.3.1.2 Animals shall be checked at least daily by a competent person or under the direct supervision of a competent person registered in terms of the relevant legislation. These checks shall ensure that all sick or injured animals are promptly identified and appropriate action is taken. There shall be provision of veterinary care and advice.

10.6.3.1.3 Special considerations shall be given to animals taken from the wild (see clause 11).

10.6.3.2 Housing and enrichment

10.6.3.2.1 Housing

10.6.3.2.1.1 The animals, except those which are naturally solitary, shall be socially housed in stable groups of compatible individuals. Incompatible animals should not be housed in the same area (see 10.6.1.2.3).

10.6.3.2.1.2 In cases where single housing is allowed subject to the approval of the AEC, the conditions shall be managed to minimize the impact of social isolation (this can be accomplished with additional environmental enrichment), the duration shall be limited to the minimum period necessary and visual, auditory, olfactory or tactile contact (or all of these) shall be maintained. The introduction or re-introduction of animals to established groups shall be carefully monitored to avoid problems of incompatibility and disrupted social relationships.

10.6.3.2.1.3 The number of animals in, and placement of, cages, pens or containers should enable the social and environmental conditions for the species to be maintained.

10.6.3.2.2 Enrichment

10.6.3.2.2.1 All animals shall be provided with space of sufficient complexity to allow expression of a wide range of normal behaviour. They shall be given a degree of control and choice over their environment to reduce stress-induced behaviour.

10.6.3.2.2.2 Establishments shall have appropriate enrichment techniques in place, to extend the range of activities available to the animals and increase their coping activities including physical exercise, foraging, manipulative and cognitive activities, as appropriate to the species.

10.6.3.2.2.3 Environmental enrichment in animal enclosures shall be adapted to the species and individual needs of the animals concerned. The enrichment strategies in establishments shall be documented and regularly reviewed and updated.

10.6.3.2.3 Animal enclosures

10.6.3.2.3.1 The animal enclosures shall not be made out of materials detrimental to the health of the animals. Their design and construction shall be such that no injury to the animals is caused.

10.6.3.2.3.2 Unless they are disposable, they shall be made from materials that will withstand cleaning and decontamination techniques.

10.6.3.2.3.3 The design of animal enclosure floors shall be adapted to the species and age of the animals and be designed to facilitate the removal of excreta.

10.6.3.3 Feeding

10.6.3.3.1 The form, content and presentation of the diet shall meet the nutritional and behavioural needs of the animal.

10.6.3.3.2 The animals' diet shall be palatable and non-contaminated. In the selection of raw materials, production, preparation and presentation of feed, establishments shall take measures to minimize chemical, physical and microbiological contamination.

10.6.3.3.3 Packing, transport and storage shall be such as to avoid contamination, deterioration or destruction. All feed hoppers, troughs or other utensils used for feeding shall be regularly cleaned and, if necessary, sterilized.

10.6.3.3.4 Each animal shall be able to access the food, with sufficient feeding space provided to limit competition.

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

10.6.3.3.6 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.

10.6.3.3.7 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.

10.6.3.3.8 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.

10.6.3.4 Watering

10.6.3.4.1 Potable water shall always be available to all animals.

10.6.3.4.2 When automatic watering systems are used, they shall be regularly checked, serviced and flushed to avoid adverse events such as flooding or dehydration. If solid-bottomed cages are used, care shall be taken to minimize the risk of flooding.

10.6.3.4.3 Provision shall be made to adapt the water supply for aquaria and tanks to the needs and tolerance limits of the individual fish, amphibian and reptile species.

10.6.3.5 Resting and sleeping areas

10.6.3.5.1 Bedding materials or sleeping structures adapted to the species shall always be provided, including nesting materials or structures for breeding animals. In cases where it may be necessary to house animals without bedding, it is to be subject to approval of the AEC and kept to a minimum.

10.6.3.5.2 Within the animal enclosure, as appropriate to the species, a solid, comfortable resting area for all animals shall be provided. All sleeping areas shall be kept clean and dry.

10.6.3.6 Handling

10.6.3.6.1 If handling or restraint is likely to cause harm, including pain, suffering, distress and lasting harm, to the animal, the use of chemical restraint (for example, sedatives) should be considered.

10.6.3.6.2 When handling or restraint is required, the animal should be conditioned to the method used, whenever possible.

10.6.3.6.3 If prolonged restraint or confinement of the animal is required as part of a project

a) methods used shall take into consideration the animal's physiological and behavioural needs, and ability to exercise,

b) the animals shall be assessed regularly by a person with veterinary, or other appropriate, qualifications who is independent of the project, and

c) if any adverse impact is detected, the animal shall be released, or the method of restraint shall be modified to minimize that impact.

10.6.3.6.4 Establishments shall set up habituation and training programmes suitable for the animals, the procedures and length of the project.

10.6.3.7 Acquisition and breeding

When animals are specifically bred for scientific purposes, the breeding programme shall be managed in accordance with current best practice to ensure the well-being of the colony, herd or flock, and all animals involved, including:

a) Maintaining, monitoring and reviewing adequate records. To allow an assessment of reproductive performance, records should include data relevant to fertility, fecundity, morbidity and mortality.

b) Ensuring that specified requirements for genetic constitution and health status are met and certified.

c) Ensuring that breeding of excess animals is avoided or minimized (see 4.7.2.7), including assessment of the details and reason for culling of animals and, when relevant, accurate and timely genotyping.

10.7 Species-specific minimum housing requirements

10.7.1 Amphibians (see annex C)

10.7.1.1 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.

10.7.1.2 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.

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

10.7.2 **Birds** (see annex D)

10.7.2.1 General

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

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

10.7.2.2 Ducks and geese

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

10.7.2.2.2 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 3). The number of birds housed shall also be determined by age, size and weight.

Table 3 — Housing areas for waterfowl

1	2	3	4
Type of waterfowl	**Area per bird**		**Minimum height**
	Group-housed m^2	Pair-housed m^2	m
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

10.7.2.3 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 4).

Table 4 — Cage and feed trough sizes for domestic fowl

1	2	3	4	5	6
Body weight	**Minimum enclosure size**	**Area per bird**		**Minimum height**	**Minimum length of feed trough per bird**
		Group-housed	**Pair-housed**		
g	m^2	m^2	m^2	cm	cm
Up to 300	1,00	0,15	0,5	30	3
301 to 600	1,00	0,2	0,5	40	7
601 to 1 200	2,00	0,3	1	50	15
1201 to 1 800	2,00	0,4	1	75	15
1 801 to 2 400	2,00	0,5	1,5	75	15
> 2 400	2,00	0,6	1,5	75	15
NOTE Adult male birds are considered in the same group as body mass >2 400.					

10.7.2.4 Quail (*Coturnix coturnix*)

10.7.2.4.1 Quail shall be group-housed in either female or mixed sex groups (see table 5).

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

Table 5 — Minimum floor space allocation for quail

1	2	3
Body weight	**Area per bird**	
g	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

10.7.2.5 Pigeons (*Columbiformes*)

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

10.7.2.6 Finches and Waxbills (*Fringillidae and Estrildidae*)

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

10.7.2.6.2 The minimum cage space for finches shall be sufficient to allow enough room for a full range of normal behaviour, including foraging, walking and flying (see table 6). An adequate number of perches shall be provided.

Table 6 — 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

10.7.2.7 Ostriches

The species-specific housing requirements for ostriches shall be as specified in SANS 994-1.

10.7.3 Cattle (see annex E)

10.7.3.1 The space allocation dimensions given in table 7 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.*

10.7.3.2 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.

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

Table 7 — Stable or pen dimensions, stocking densities and minimum floor area requirements for cattle

1	2	3	4
Body weight	**Minimum floor area per animal**		**Minimum length of feed rack or trough per head**
kg	Group-housed m^2	Single-housed m^2	m
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

10.7.4 **Cephalopods** (see annex F)

A tank of 1 m diameter and 0,6 m depth shall be provided for 5 to 10 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.

10.7.5 **Dogs and cats** (see annex G)

10.7.5.1 **Sleeping areas**

10.7.5.1.1 All dog kennels and cat cages should be provided with a raised sleeping area. Cats should be provided with one raised sleeping area per cat. In a multi cat unit the raised areas should be at different heights.

10.7.5.1.2 Facilities should consider providing beds and bedding for animals, both for comfort and for environmental enrichment. However, the risk of contamination with test items or test systems may dictate that the use of bedding is contra-indicated during certain studies.

10.7.5.2 **Pen and cage sizes — Dogs** (see tables 8 to 11 (inclusive)).

10.7.5.2.1 The 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.

10.7.5.2.2 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.

10.7.5.2.3 **Dog cages for scientific purposes**

Table 8 shows the recommended minimum sizes for the housing of dogs for scientific purposes:

Table 8 — Dog cages used in studies

1	2	3	4	5
Body weight kg	**Minimum enclosure size** m^2	**Minimum floor area for one or two animals** m^2	**For each additional animal add a minimum of** m^2	**Minimum height** m
up to 20	4	4	2	2
over 20	8	8	4	2

10.7.5.2.4 **Breeding pens for dogs**

Table 9 shows the recommended minimum sizes for breeding bitches and their litters up to weaning. The exercise runs should be longer than wide to prevent dogs from running in circles. The ideal run of 12 m^2 can be 1,5 m × 8 m.

Table 9 — Dog breeding pens — pregnant bitches and litters

1	2	3	4
Body weight of bitch kg	**Minimum sleeping quarters** m^2	**Minimum exercise run floor area** m^2	**Minimum height** m
Any	2,5	12	2

Table 10 — Dog: post weaned stock

1	2	3	4	5
Body weight kg	**Minimum enclosure size** m^2	**Minimum floor area per animal** m^2	**Maximum number of animals per enclosure**	**Minimum height** m
up to 5	4	0,5	8	2
over 5 to 10	4	1,0	4	2
over 10 to 15	4	1,5	3	2
over 15 to 20	4	2,0	2	2
over 20	8	4,0	2	2

10.7.5.2.5 Communal housing

Table 11 shows the recommended minimum sizes for exercise runs and separate sleeping quarters when dogs are not in studies as result of a compulsory ethics resting period or a test item washout period. Exercise runs should be longer than wide to prevent dogs from running in circles. The ideal run of 28 m^2 can be 1,5 m × 19 m.

Table 11 — Communal housing – dog exercise runs

1	2	3	4	5
Body weight of bitch kg	**Minimum sleeping quarters** m^2	**Minimum exercise run floor area** m^2	**Maximum number of animals per run**	**Minimum height** m
Up to 20	4,5	28	3	2
Over 20	4,5	28	2	2

10.7.5.3 Pen and cage size — Cats (see table 12)

10.7.5.3.1 Cat cages for scientific purposes

Table 12 shows the recommended minimum sizes for the housing of cats for scientific purposes.

Table 12 — Cat cages used in studies

1	2	3	4
Enclosure	**Minimum enclosure size**[a] m^2	**Shelves** m^2	**Minimum height** m
Minimum for one adult animal	1,5	0,5	2
For each additional animal add a minimum of (m^2)	0,75	0,25	–
[a] Floor area excluding shelves.			

10.7.5.3.2 Cat breeding pens – queens and litters

Table 13 shows the recommended minimum sizes for breeding queens and their litters up to weaning.

Table 13 — Breeding queens and their litter

1	2	3	4
Enclosure	**Minimum enclosure size**[a] m^2	**Shelves** m^2	**Minimum height** m
Minimum for one adult animal and her litter	4,5	0,5	2
[a] Floor area excluding shelves. A separate shelf area shall be provided to allow the queen some personal space distant from the litter.			

10.7.5.3.3 Communal housing

10.7.5.3.3.1 Cats shall have sleeping quarters with access to an exercise area. The exercise area will have raised areas (shelves and or tree stumps) and scratch poles (see table 14).

Table 14 — Cat exercise runs

1	2	3	4	5
Type of cat	**Sleeping quarters**[a] m^2	**Exercise area** m^2	**Maximum number of adult animals**	**Height** m
Adult cats	2	4	3	2
[a] Floor area excluding shelves.				

10.7.5.3.3.2 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.

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

10.7.5.3.3.4 Areas for feeding and for litter trays shall not be less than 0,5 m apart and shall not be interchanged.

10.7.6 **Fish** (see annex H)

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.

10.7.7 **Horses** (see annex I)

10.7.7.1 The space allocation dimensions given in table 15 are for in-house holding facilities for horses.

Table 15 — 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 or horse
Group box	> 200	7,5 or horse
Foaling box	–	15

10.7.7.2 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.

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

10.7.8 **Non-human primates (baboons, vervet and rhesus monkeys)** (see annex J)

10.7.8.1 The space allocation dimensions for in-house holding facilities for non-human primates are given in tables 16 and 17. Table J.1 gives the species maximum morphological parameters taken into consideration in the design of cages and perches for non-human primates.

10.7.8.2 For macaques and vervets, separation from the mother shall not take place before eight months of age.

Table 16 — Guidelines for cage sizes for macaques and vervets[a]

1	2	3	4	5
Type of animal	**Minimum enclosure size** m^2	**Minimum enclosure volume** m^3	**Minimum volume per animal** m^3	**Minimum enclosure height** m
Animals less than three years of age[b]	4,0	7,2	2,4	1,8
Animals from 3 years of age[c]	4,0	7,2	1,44	1,8
Animals held for breeding purposes[d]	–	–	3,5	2,0

[a] Animals shall be kept singly only in exceptional circumstances.

[b] An enclosure of minimum dimensions may hold up to three animals.

[c] An enclosure of minimum dimensions may hold up to two animals.

[d] In breeding colonies no additional space or volume allowance is required for young animals up to two years of age housed with their mother.

Table 17— Guidelines for cage sizes for baboons[a]

1	2	3
Weight of primate kg	**Minimum floor area** m^2	**Minimum enclosure height** m
7 to 9	4,00	1,80
9 to 15	4,00	1,80
15 to 25	7,00	1,80
>30	7,00	2,10
Use decimal commas in column 2.		
NOTE 1 For breeding purposes the minimum breeding space is 12 m^3 per mother and offspring less than two years of age.		
NOTE 2 When deciding on cage sizes for baboons, the same considerations as for vervet monkeys (see J.7.6.4) apply, except that a much larger cage is used, with a higher and larger resting perch.		
[a] Animals shall be kept singly only in exceptional circumstances.		

10.7.8.3 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).

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

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

10.7.9 **Pigs** (see annex K)

10.7.9.1 For space guidelines on the housing of pigs, refer to National Research Council. 2012. *Nutrient Requirements of Swine: Models for Estimating Nutrient Requirements of Pigs Case studies* and the ECD (European Commission Directive, 2010). The recommendation should be rather used as guidelines and common sense should prevail.

10.7.9.2 The animals should be provided with housing, environment, degree of movement, food, water and care which are appropriate to their health and well-being. The five freedoms should be applied. Restrictions to the physiological and behavioural needs of the animals should be limited to the absolute minimum. In general, less space is given to swine in agricultural settings than what is recommended for research swine.

Table 18 — Space recommendations (Bollen *et.al*., 2010)

1	2	3	4
Number of swine per enclosure	**Weight** kg	**Floor area per animal** ft^2	**Floor area per animal** m^2
1	<15	8	0,72
	15 to 25	12	1,08
	25 to 50	15	1,35
	50 to 100	24	2,16
	100 to 200	48	4,32
	>200	>60	>5,40
2 to 5	<25	6	0,54
	25 to 50	10	0,90
	50 to 100	20	1,80
	100 to 200	40	3,60
	>200	>52	>4,68
>5	<25	6	0,54
	25-50	9	0,81
	50 to 100	18	1,62
	100 to 200	36	3,24
	>200	>48	>4,32
Space recommendations adapted from *The Laboratory Swine, second edition,* Bollen *et.al*., 2010.			

10.7.9.3 Individually housed animals should have visual, olfactorial and auditory contact with other swine, to prevent social deprivation. Environmental enrichment should be provided so that the needs of chewing and rooting can be met. Chains, hayracks, broom heads or nylon brushes, heavy plastic balls, or plastic turf mats should be given to the animals for a limited period daily.

10.7.10 **Rabbits and guinea pigs** (see annex L)

10.7.10.1 The minimum pen space for rabbits and guinea pigs shall be of enough room to allow a full range of normal behaviour (see tables 19 and 20) note that cage floor area for animals in groups shall be not less than that for single housed animals. The dimensions provided in tables 19 and 20 are the internal floor area available to the animals. Shelf areas may be included if the animals can utilized that space below and above.

10.7.10.2 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.

10.7.10.3 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. Areas that provide complete visual breaks from other animals are necessary for the animals to be able to control their own environment, for example, a refuge or hutch in the animal enclosure.

10.7.10.4 Bedding may be withheld as part of time-mating practices.

Table 19 — Cage or pen dimensions, stocking densities and minimum housing requirements for breeding rabbits and guinea pigs (including litters)

1	2	3	4
Breeding category	**Minimum floor area** cm^2	**Minimum cage height** cm	**Nest box space (additional)** cm^2
Rabbits			
Doe and litter <3 kg	4 000	45	1 000
Doe and litter >3 kg	6 400	45	1 400
Guinea pigs			
Mother and litter Monogamous pair and litter	2 500	23	–
For each additional female in harem	1 000	23	–

Table 20 — Cage dimensions, stocking densities and minimum housing requirements for non-breeding rabbits and guinea pigs. (Directorate, 2015)

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	3 500	45	3 000	45
3,1 to 3,5	4 000	45	4 000	45
3,6 to 5,0	4 500	45	4 000	45
5.1 to 6,0	5 400	60	5 400	45
>6,0	6 000	45	6 000	45

Table 20 *(concluded)*

Guinea pigs				
Body weight	**Group-housed**		**Single-housed**	
	Minimum floor space	**Minimum cage height**	**Minimum floor space**	**Minimum cage height**
g	cm^2/animal	cm	cm^2/animal	cm
< 150	200	20	700	20
151 to 250	300	20	700	20
251 to 350	400	20	900	23
351 to 450	500	20	900	23
451 to 550	700	23	900	23
>550	700	23	1 000	23
>650g	900	23	1 250	23
Changes based on the *Code of practice for the housing and care of animals used in scientific procedures –Part 2: 5. Tables based upon the RS/UFAW*.				

10.7.11 Rodents (mice, rats and hamsters) (see annex M)

10.7.11.1 The minimum pen space for rodents shall be of enough room to allow a full range of normal behaviour (see tables 21 and 22).

10.7.11.2 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.

Table 21 — 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 22 — 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

10.7.12 Sheep and goats (see annex N)

10.7.12.1 The space allocation dimensions given in table 23 are for in-house holding facilities for sheep and goats. These are approximate guidelines that need to be adapted in accordance with the circumstances. 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 23 — 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

Table 23 *(concluded)*

1	2	3
Type of animal	**Minimum floor area per animal**	
	Group-housed m^2	Single-housed m^2
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.		

10.7.12.2 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.

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

10.7.13 Terrestrial reptiles (see annex O)

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

11 Wildlife

11.1 General

This clause covers the requirements for any projects or teaching activities performed on wildlife, such projects and activities include but are not limited to tracking, observational studies, capture of animals from the wild, control of feral animals etc.

11.2 General considerations

11.2.1 Facilities working with wildlife are required to have applicable reference texts such as *The Capture and Care Manual*, 1993. Edited by McKenzie, A. The Wildlife Decision Support Services and South African Veterinary Foundation, Pretoria. or Fowler, M. E. 2011. *Restraint and handling of wild and domestic animals*. John Wiley & Sons or similar such texts.

11.2.2 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.

11.2.3 The well-being of wildlife shall be supported and safeguarded by:

a) using methods, techniques and equipment that

1) are appropriated for the species and situation, and the purpose and aims of the project or activity, performed in accordance with the accepted current standards and backed up by scientific evidence, and

2) minimize the risk of transmission of disease, and direct and indirect disturbance to the habitat.

b) avoiding or minimizing harm, including pain, suffering, distress and lasting harm

1) to target and non-target species,

2) to dependent young, and

3) from indirect effects arising from impact on the habitat, environment and any effects on biodiversity.

11.2.4 Animals taken from the wild shall not be used in procedures, except for the conditions stated in 11.3.2.

11.2.5 For research or procedures performed in zoological gardens or aquariums, the zoological gardens or aquariums shall be operated and managed in accordance with SANS 10379.

11.3 Provisions on the use of certain animals in procedures

11.3.1 Threatened and Endangered species

Specimens of those Threatened and Endangered species listed in relevant national regulation (see foreword), the Convention on International Trade in Threatened and Endangered Species of Wild Fauna and Flora (CITES) or the IUCN red list, on the protection of species of wild fauna and flora by regulating trade therein:

a) shall not be used in procedures, with the exception of those procedures meeting the following conditions or the procedure having the purposes referred to in the following points;

1) the avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality or their effects in human beings, animals or plants, that cannot be achieved by the use of another species;

2) the development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feed-stuffs and other substances or products that cannot be achieved by the use of another species;

3) research aimed at improving knowledge required for the preservation of the species, or of isolated populations, subspecies or cryptic evolutionary lineages that may be on conservation concern;

b) Paragraph 1 (11.3.1 introduction paragraph) shall not apply to any species of non-human primates, except for a)(3) (see 5.8.3.2(e)(2)).

11.3.2 Animals taken from the wild

11.3.2.1 The AEC may grant exemptions from 11.2.4 on the basis of scientific justification to the effect that the purpose of the procedure cannot be achieved by the use of an animal which has been bred for use in procedures.

11.3.2.2 The capture of animals in the wild shall be carried out only by competent persons using methods which do not cause the animals avoidable pain, suffering, distress or lasting harm. Any use and capture or trapping of animals in the wild shall consider and safeguard the interests of not only

the target animals but non target animals. Any animal found, at or after capture, to be injured or in poor health shall be examined by a veterinarian or another competent person under supervision of a veterinarian and action shall be taken to minimize the suffering of the animal, any such instances are to be reported to the AEC. The AEC may grant exemptions from the requirement of taking action to minimize the suffering of the animal if there is scientific justification.

11.3.2.3 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.

11.3.2.4 Where animals are captured from the wild and need rehabilitation before their return to the environment, provisions of ARP 1048 shall apply.

11.3.3 Stray and feral animals of domestic species

11.3.3.1 This standard applies equally to feral animals.

11.3.3.2 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 5.8.3.2(d) to 5.8.3.2(e)).

11.3.3.3 Relevant regulatory requirements, specific literature, *"Restraint and Handling of wild and domestic animals"*, Fowler ME, standards such as SANS 10331, shall be consulted relating to the capture, holding and transport of stray and feral animals.

11.3.3.4 Since the background of stray and feral animals of domestic species is not known, and since capture and placement into establishments increases distress for such animals, they should not, as a general rule, be used in procedures.

11.3.3.5 The AEC may only grant exemptions from 11.3.3.4 subject to the conditions in 10.3.5.

11.3.4 Studies involving vertebrate pest animals

11.3.4.1 The principles of this standard shall be applied equally to animals that are considered to be pests.

11.3.4.2 Captive feral and pest species shall be killed humanely unless the aims of a project require their release.

11.4 Capture and handling

11.4.1 Many species of wildlife are protected by provincial ordinances. Provincial 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 adhered to. All capture and handling shall be approved by the AEC.

11.4.2 Capture is stressful to animals, and steps shall be taken to minimize the disruption of the population and any stress or 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 stress or distress following capture at a frequency and level which will protect the welfare of the animals and remedial steps taken if necessary.

11.4.3 To minimize the risk of injury or stress-induced disease, procedures for the capture and handling of wildlife shall include

a) the involvement of a sufficient number of competent people to restrain animals in a quiet environment and prevent injury to animals and handlers,
b) chemical restraint (for example, sedatives) where appropriate, if the period of handling is likely to cause harm, including pain, suffering, distress and lasting harm, to animals (scheduled substances are regulated by the relevant national department (see foreword),

c) restraint and handling of animals for the minimum time needed to achieve the purpose and aims of the project or activity, and

d) making provisions for captured animals that are ill or injured, including treatment of pain, suffering, distress and lasting harm.

11.5 Use of traps

11.5.1 If trapping is used to capture wildlife, the well-being of both target and non-target animals shall be considered and documented by

a) selecting a trap that is suited to the species of an appropriate size and depth and the circumstances, and designed to ensure protection of trapped animals from injury, predators, parasites, deprivation of food and water and environmental extremes (such as dehydration and drowning),

b) frequent monitoring of traps to minimize the time animals will spend in traps, and to avoid or minimize adverse impacts on trapped animals,

c) minimizing the number of days of continuous trapping within an area, and removing or deactivating traps that are not in use or are no longer required,

d) minimizing the potential adverse impact caused by disrupting social structure, and adverse impacts on dependent young (for example, by avoiding trapping in the breeding season), and

e) minimizing the numbers of non-target species that are trapped, and implementing a management plan for captured non-target species to ensure their well-being, and release or ensure that they are humanely killed.

11.5.2 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, *"Restraint and Handling of wild and domestic animals"*, Fowler ME and SANS10331 shall be consulted.

11.5.3 Wet pitfall traps shall not be used to capture vertebrate animals. If wet pitfall traps are used to capture invertebrates, they shall be managed and monitored to minimize the inadvertent capture of vertebrates, including by locating the trap where vertebrate entry is unlikely and using the smallest possible trap diameter.

11.5.4 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.

11.5.5 The selling, inspection and laying of traps and other devices for the purposes of capturing and destroying animals is subject to provisions under the relevant national legislation (see foreword).

11.5.6 A wide variety of non-trap capture techniques can also be used in field studies. Similar principles apply as those detailed in 8.3.1.2 for traps. The skill of the operator is essential to ensure minimal impact on target and non-target species.

11.6 Transport, holding and release

11.6.1 Transport

11.6.1.1 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 10.2, 10.3 and 10.4 apply, and particular reference may be made to, but not limited to SANS 1884-2, SANS 1884-3, SANS 10331 and the wildlife section of the IATA regulations, to *Restraint and handling of wild and domestic animals*, Fowler ME.

11.6.1.2 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,

f) administering sedatives and tranquillizers, if appropriate, by suitably trained persons and in accordance with the relevant national legislation (see foreword), and

g) resting the animals, where relevant.

11.6.1.3 Transport methods and arrangements shall be

a) documented and approved by the AEC,

b) be appropriate for the species and the circumstances,

c) minimize harm, including pain, suffering, distress and lasting harm, arising from factors such as containment, movement, noise, disruption of social groups, and changes in the environment and personnel,

d) ensure that animals are

1) provided with appropriate food and water when necessary,

2) provided with the physical and social environment appropriate for the species,

3) protected from, and treated for, injury and disease.

11.6.1.4 For further information on the transportation of animals also see 10.2.1; 10.2.5 to 10.2.7 (inclusive).

11.6.2 Holding and release

11.6.2.1 If animals are to be held in captivity, 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. If animals are to be released, all possible steps shall be taken to avoid their becoming habituated to human activity.

11.6.2.2 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.

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

11.6.2.4 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.

11.6.2.5 Procedures for any release of wildlife shall ensure that:

a) Release occurs at the site of capture, unless otherwise approved by the AEC.

b) The timing of release coincides with the period of usual activity for the species, unless safety of the animals is assured by other means, such as release into appropriate cover.

c) Animals are protected from injury and predation at the time of their release.

d) Animals that have been sedated or anaesthetised have recovered to full consciousness before their release. During their recovery, animals should be held in an appropriate area where they can maintain normal body temperature and are protected from injury and predation.

11.6.3 Housing and care

11.6.3.1 Animals shall be provided with housing, physical and social environmental conditions, food, water and care to meet species-specific or strain-specific physical and behavioural needs. If the requirements of a project or activity preclude or modify these conditions, special ethics consideration and specific AEC approval are required (see 4.4.2 and 6.2(a)).

11.6.3.2 Facilities shall be appropriately staffed, designed, constructed, equipped, maintained and managed to achieve a high standard of animal care. Facilities shall be suitable for the type of animals kept, the aims of the activities undertaken and environmental enrichment provided.

11.6.3.3 Animals held outdoors shall be protected from adverse environmental conditions and predation, and provided with access to adequate shelter, food and water.

11.6.3.4 The housing and care of animals that are administered infectious organisms shall take into account risks to other animals and to humans, and appropriate procedures to minimize such risks shall be implemented.

11.6.3.5 Animals caught from wild populations and brought into captivity should be housed in isolation from other animals to prevent spread of disease. Zoonotic implications and inter-species disease concerns shall be considered. Incompatible species should not be housed in close proximity, see 10.6.1.2.3.

11.7 Tracking the movement of wildlife

11.7.1 When devices are used to track the movement of wildlife, the weight, design and positioning of attached devices shall minimize interference with the normal survival requirements of the animal.

11.7.2 External devices no longer in use should ideally be removed so as not to interfere with normal survival. Animals fitted with tracking devices for scientific purposes shall have the AEC approval.

11.8 Interference activities

Interference activities such as call playback, spotlighting, tilling, rock turning, investigating a nest box and disturbing nest sites shall be conducted in a manner that minimizes any risk to the well-being of the wildlife, with careful considerations with regard to other wildlife biodiversity and disturbance; informed consent from owners is a necessity.

11.9 Voucher specimens

Alternatives to collecting animals as voucher specimens (for example, tissue specimens, digital photography) shall be considered, where appropriate. When animals are collected as voucher specimens

a) the number taken shall be the minimum required for identification or to establish distribution,

b) 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,

c) 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

d) proper documentation of the specimens, including reasons for collection, is essential and data shall be maintained together with the specimens.

11.10 Identification of animals

11.10.1 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.

11.10.2 Methods used to identify animals shall:

a) Be appropriate for the species and the circumstances.

b) Be compatible with the purpose and aims of the project or activity.

c) Involve non-invasive methods whenever possible. The use of invasive methods shall conform to 6.4.2.

d) Cause the least harm, including pain, suffering, distress and lasting harm, to the animals.

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

11.10.4 Identification methods shall be approved by the AEC.

11.11 Field techniques

11.11.1 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 (use of which is regulated by relevant regulatory authority). Such procedures could include identification (for example, leg banding, ear tagging, micro-chipping and radio-tracking devices), examination, measurement, and sampling (for example, hair, feathers, scales, blood and stomach contents of birds).

11.11.2 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.

11.12 Wildlife interaction studies

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

11.12.2 The primary ethics 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.

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

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

11.12.5 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.

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

12.1 General

12.1.1 This clause covers the requirements for the use of farm animals for scientific studies and teaching activities. The inclusion of this clause in the code does not preclude the requirements of clause 10.

NOTE Teaching includes demonstrations.

12.1.2 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 the AEC approval is required for the use of farm animals.

12.2 General requirements

12.2.1 Unless specifically exempted by the AEC, the care of farm animals managed by institutions shall at least comply with available ethics and scientific animal husbandry practices.

12.2.2 The 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 12.2.3.

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

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.

12.2.3 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 regulatory body (see foreword) to carry out the procedure.

12.2.4 An annual return detailing the number of all animals maintained at the institution and the purposes for which they are kept shall be provided to the AEC (see 5.5.3.6(a).

12.3 The AEC applications

12.3.1 The AEC approval is required for teaching or demonstrating routine procedures not covered in 12.2.3.

12.3.2 To simplify the 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, responsibility for the use of animals and the replacement of animals by alternative, non-animal techniques should be included, where possible.

12.3.3 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.

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

12.4 Teaching and demonstration requirements for all farm animals

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

12.4.1 Facilities shall be available to treat animals that might be injured. Treatment might range from a minor procedure to euthanasia. Access to a veterinarian shall be available. Facilities shall be available for the continued treatment of animals should the procedure being demonstrated or taught require analgesia or other treatments to be continued in the days following the demonstration or event.

12.4.2 If animals are to be handled then there shall be a competent person present to protect animals from injury or distress or stress or lasting harm. Suitable facilities shall be available for handling the animals. Animals that do not adapt to the situation shall be removed.

12.4.3 Detailed guidelines and complete animal care records shall be available for inspection at all times. Records of animal use should include the monitoring of animals after procedures, the monitoring of the number of times an individual animal is used for a procedure and the animal's response to such use. If the AEC stipulated limitations on the number of repetitions per animal, records to show this repetitions were adhered to shall be kept. The AEC protocols should include monitoring requirements and endpoints for procedures that will minimize any distress the animal may experience during these procedures.

12.4.4 Animals shall not be held for longer than necessary. Animals are not to be left in holding facilities (for example, crush or neck clamp) without supervision.

12.4.5 When teaching and demonstrations are not one day events, arrangements for regular and on-going monitoring of the animals shall be made, including weekends and holding facilities shall be secure against human or animal interference. Such monitoring and frequencies shall be outlined in the AEC protocol and approved. When teaching and demonstrations are one day events, arrangements for on-going monitoring of animals (should it be necessary after that day) shall be made and outlined in the AEC protocol.

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

13.1 General

This clause refers to the special ethics 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 emphasize the principles most relevant to schools and tertiary institutions.

13.2 General principles

13.2.1 The animals shall be used for teaching activities only when there are no suitable alternatives for achieving the educational objectives.

13.2.2 All teaching activities which involve the use of animals shall have approval by the 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.

13.2.3 Students shall be given the opportunity to discuss the ethics, 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.

13.3 Responsibilities of lecturers and teachers

13.3.1 The teacher or the 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 national legislation (see foreword), standards and guidelines (see clause 4);

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 the 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.

13.3.2 The lecturer or the 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.

13.3.3 Persons supervising students who are undertaking training in research shall ensure that before using animals, the students receive appropriate instruction in the ethics and legal responsibilities involved in the use of animals for scientific purposes, as well as in the appropriate methods for animal care and use. The person supervising such students shall be responsible for the welfare of the animals used by those students.

13.4 Animals used in primary and secondary schools

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

13.4.2 The head of the school shall ultimately be responsible for ensuring compliance with the requirements in 13.4.

13.4.3 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 bio-hazardous materials.

13.4.4 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.

13.4.5 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.

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

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

13.4.8 The 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
(normative)

Non-species specific animal housing and husbandry requirements

A.1 General care of animals

A.1.1 Facilities include the buildings, cages (including isolation cages for sick animals), pens, stalls or pastures in which animals are kept.

A.1.2 The institutions, the researchers, the teachers and the 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.

A.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.

A.1.4 Outdoor holding facilities and shelters 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. (A sheltered housing facility is a facility that provides shelter, protection from the elements, and protection from temperature extremes at all times. It can consist of runs or pens in a totally enclosed building which has no climate control or indoor-outdoor runs with the indoor runs in a totally enclosed building without climate control).

A.1.5 Incompatible species (such as prey and predator) and species requiring different environmental conditions are not to be housed in the same room (if prey and predator species, the animals are not to be seen within sight. Smell and sound of one another under exceptional circumstances approved by the AEC.)

A.2 Indoor housing (see annexes C to O (inclusive))

A.2.1 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.

A.2.2 Buildings shall be designed, operated and maintained to:

a) Control environmental factors appropriately.

b) Exclude pest animals,

c) Limit contamination associated with the keeping of animals, the delivery of food, water and bedding, and the entry of people and other animals.

d) Achieve the effective control of pest animals. A pest control programme that covers all areas shall be in place.

e) Provide adequate storage facilities to separate feed, bedding, chemicals, clean cages, drugs, equipment and records, etc..

f) Provide a separate storage area for flammables that is lockable and secure with access control, and that complies with the relevant national legislation (see foreword).

A.2.3 Buildings shall be maintained in good repair and kept clean and tidy. Walls and floors shall be constructed of durable materials that can be cleaned and disinfected readily.

A.2.4 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.

A.2.5 Detergents, disinfectants, deodorants and pesticides might contaminate the animals' environment. Choice of agents shall be made in consultation with the researchers.

A.2.6 There shall be a reticulated water supply and proper facilities for drainage, if appropriate.

A.2.7 There shall be adequate contingency plans to cover emergencies such as the breakdown of lighting, heating or cooling, and fire or flood.

A2.8 Precautions shall be taken against the entry of unauthorized persons or products (or both).

A.2.9 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.

A.2.10 Air exchange, temperature, humidity, light, noise and vibration shall be maintained within limits compatible with the health and well-being 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.

A.3 Environmental factors

A.3.1 Animals shall be provided with environmental conditions that suit their biological needs unless otherwise approved by the AEC for the purposes of a study.

A.3.2 Noxious odours, particularly ammonia, shall be kept to a level compatible with the health and comfort of the animals and staff. 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.

NOTE 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.

A.3.3 The environmental factors in A.3.2 can 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.

A.3.4 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.

A.3.5 Staff shall be trained to avoid practices that generate excessive and unnecessary noise.

NOTE Separation of human and animal areas will minimize disturbances.

A.3.6 Procedures, or manipulations on animals, shall be conducted in separate designated areas, well away from other animals.

A.4 Food and water

A.4.1 Animals shall receive, and be able to access, appropriate, uncontaminated, nutritionally adequate food of a quantity and composition that maintain normal growth of immature animals and normal weight of adult animals, and meet the requirements of pregnancy, lactation or other conditions.

A.4.2 Clean, fresh drinking water shall be available at all times, as suitable for the species.

A.4.3 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.

A.4.4 Each animal shall be able to access the food, with sufficient feeding space provided to limit competition.

A.4.5 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.

A.5 Pens, cages and containers

A.5.1 Animal housing shall be designed and managed to meet species-specific needs. Pens, cages and containers shall be constructed and maintained to ensure the comfort and well-being 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 specimens).

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.

i) The passages' cleaning and sanitizing programme.

A.5.2 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' well-being; and

h) be compatible with the behavioural needs of the species.

A.5.3 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 the AEC approval.

A.5.4 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.

A.5.5 The AEC and the 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.

A.5.6 The number of animals in, and placement of, cages, pens or containers should enable the social and environmental conditions for the species to be maintained.

A.5.7 If an animal of a species that normally lives in social groups is to be housed in isolation or separated from a group, the duration of such housing conditions shall be minimized (see 6.3.3.1.8 and 6.4.8.2). The animal should be able to see, hear and smell animals of the same species unless such contact is precluded by the requirements of the activity.

A.6 Environmental enrichment

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

A.6.1 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.

A.6.2 All animals shall be provided with space of sufficient complexity to allow expression of a wide range of normal behaviour. They shall be given a degree of control and choice over their environment to reduce stress-induced behaviour. Establishments shall have appropriate enrichment techniques in place, to extend the range of activities available to the animals and increase their coping activities including physical exercise, foraging, manipulative and cognitive activities, as appropriate to the species. Environmental enrichment in animal enclosures shall be adapted to the species and individual needs of the animals concerned. The enrichment strategies in establishments shall be regularly reviewed and updated.

Annex B
(informative)

Genetic and microbiological status of laboratory animals

B.1 No formal standards have been created for South African purposes. It is, therefore, recommended that the international standards should be referred to. Good guidance can be found in the annex references (listed per species) and in the Bibliography Annex on Genetic and microbiological status of laboratory animals.

B.2 Facilities should perform regular testing of the animals, and test as thoroughly as possible. The extent of testing may be dictated by the research involved.

Annex C
(informative)

Care and management of amphibians (frogs (*xenopus laevis*))

C.1 General

C.1.1 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.

C.1.2 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.

C.1.3 The types of amphibians generally encountered in laboratories are frogs, toads and salamanders.

C.1.4 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.

C.2 Background information

C.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.

C.2.2 Species characteristics and biological information

C.2.2.1 *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.

C.2.2.2 These animals have very sensitive nerve endings along the body (lateral line) and therefore need to be handled gently.

C.2.2.3 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.

C.2.2.4 Large fat bodies are attached to each kidney to provide energy during hibernation and reproduction.

C.2.2.5 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.

C.2.2.6 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 two years to three 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.

C.2.2.7 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.

C.2.2.8 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.

C.2.2.9 Their diet is mainly carnivorous as they eat carrion, small worms, insects, and each other.

C.2.2.10 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 should come to the surface to breathe or they will drown.

C.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.

C.3 Supply and transport

C.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.

C.3.2 Transport

C.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.

C.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.

C.3.2.3 For frogs being transported by air, the IATA standards and recommendations should be adhered to (see 10.4.2). Flights should be planned to ensure the journey times are of the minimum duration possible.

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

C.3.3 Quarantine

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

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

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

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

C.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".

C.3.3.6 Appropriate action for detecting and treating for Chytrid fungus should be taken. The consequences of spreading this disease is serious, more so than ″red leg″.

C.4 Housing and care

C.4.1 Humane handling

C.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.

C.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.

C.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.

C.4.2 Lighting

C.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 or 12 h light or dark cycle is recommended.

C.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.

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

C.4.3 Thermoregulation

C.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.

C.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.

C.4.4 Water provision and quality

C.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 10.7.1.2).

C.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.

C.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.

C.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.

C.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.

C.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.

C.4.4.7 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 C.1 may be used for water quality control.

Table C.1 — Guidelines for water quality control

1	2
Determinands	**Allowable quantity**
Alkalinity and hardness as $CaCO_3$	50 mg/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 cold water	 > 5 mg/L > 8 mg/L

C.4.4.8 Water samples can be tested periodically at a laboratory to ensure constant quality.

C.4.4.9 Avoid galvanized or copper piping. Zinc may be leached from galvanized pipes and copper from copper or brass pipes.

C.4.5 Tank housing

C.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.

C.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.

C.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.

C.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.

C.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.

C.4.6 Sanitation and disinfection

C.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.

C.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.

C.4.6.3 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.

C.4.7 Identification methods and records

C.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.

C.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.

C.4.7.3 Preferred methods of identification are photographic records as well as skin pattern diagrams.

C.4.8 Group housing

C.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.

C.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 × 27 cm × 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.

C.4.8.3 On average, groups of five to twenty frogs per tank are held in the laboratory.

C.5 Handling techniques

C.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.

C.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.

C.5.2 Powdered gloves should not be used. Gloves should be moistened with water before handling frogs.

C.5.4 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.

C.6 **Nutrition** (Food types and feeding regimen)

C.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).

C.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.

C.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.

C.6.4 Attacking each other at feeding time usually indicates overcrowding or frogs being underfed.

C.6.5 It is important to watch animals eating. Those not feeding might be sick and should be removed from the tank.

C.6.6 Frog tadpoles are herbivores and filter feeders. They will begin feeding on suspended food particles in about 10 days. 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.

C.6.7 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.

C.6.8 Frogs and other *anura* do not drink but absorb water through a highly permeable specialized area in the pelvis region.

C.7 Environmental enrichment

C.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 should have smooth and rounded edges to prevent injury.

C.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.

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

C.8 Health assessment and disease prevention

C.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.

C.8.2 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.

C.8.3 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.

C.8.4 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.

C.8.5 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.

C.9 Common diseases

C.9.1 Bacterial diseases

C.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.

C.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.

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

C.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. There should be appropriate action for detecting and treating for Chytrid fungus as the consequences of spreading this disease is serious, more so than ″red leg″.

C.9.3 Parasitic diseases

C.9.3.1 Amphibians have a large number of parasites which generally do not cause problems. Quarantine procedures should include routine anthelmintic treatments.

C.9.3.2 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.

C.9.4 Protozoans

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

C.9.5 Non-infectious diseases

C.9.5.1 Neoplasia occurs but is not common.

C.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.

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

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

C.10.1 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.

C.10.2 Anaesthetics, antibiotics and anthelmintics can be effectively administered when used in the water or dropped onto the skin.

Table C.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
Benzalkoniun 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
[a] To be prescribed by a qualified veterinarian.		

Table C.2 *(concluded)*

1	2	3
Drug	**Recommended dose**[a]	**Recommended route and dose interval**[a]
Ketoconozole	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.		

C.11 Antiparasitics (see table C.3)

Table C.3 — Recommended doses of antiparasites 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.		

C.12 Scientific procedures

C.12.1 General

C.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.

C.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).

C.12.2 Breeding and reproduction

C.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.

C.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.

C.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.

C.12.2.4 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 Gonadotropic 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.

C.12.2.5 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.

C.12.2.6 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.

C.12.3 Egg-harvesting and care

C.12.3.1 General

During egg development, the water should 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.

C.12.3.2 Oocyte collection

C.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.

C.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.

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

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

C.12.4 Hatching larvae or oocytes

C.12.4.1 The hatching larvae or oocytes generally stay attached to the jelly while the yolk sacs are absorbed.

C.12.4.2 Feeding will commence in 5 days to 10 days.

C.12.4.3 Tanks should be kept clean and aerated.

C.12.5 Care and rearing of tadpoles

C.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 C.4, or in 5 L when metamorphosis begins. For best results, 50 % of the water should be changed daily.

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

Table C.4 — Pond water formula

1	2
Stock solution A	**Stock solution B**
175 g NaCl	5 g $HaHCO_3$
35 g $CaCl_2$	–
2 L distilled water	2 L distilled water

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

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

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

C.12.6 Hibernation

C.12.6.1 Hibernating frogs should not be fed.

C.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.

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

C.13 Blood sampling

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

C.13.2 Blood sampling 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.

C.14 Injections and sites

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

C.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.

C.14.3 The thigh muscles can be used for intramuscular injections.

C.14.4 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.

C.14.5 Gavage by stomach tube is very stressful to amphibians and is not recommended.

C.15 Analgesia and anaesthesia

C.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.

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

C.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.

C.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 C.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	50 m 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.		

C.16 Euthanasia

C.16.1 General

C.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.

C.16.2 Sodium pentobarbital (60 mg/kg to 100 mg/kg) is injected into the dorsal lymph sac or IP.

C.16.3 Pithing, a process of decerebration followed by spinalization, is not acceptable. Decapitation should be preceded by chemical restraint (as given in C.16.1).

C.16.4 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.

C.17 Training of personnel

C.17.1 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.

C.17.2 Inappropriate handling will impact on the animals' well-being and affect scientific results. Training of personnel should be species-specific.

Annex D
(informative)

Care and management of birds

D.1 General

D.1.1 All birds share the same basic anatomy and physiology, despite their diverse range of adaptations for locomotion and feeding.

D.1.2 The social systems of birds vary from highly social to predominantly solitary and therefore the conditions under which they are maintained should reflect the social system of a particular species. Social species 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.

D.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.

D.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.

D.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 Threatened and Endangered Species (CITES) of Wild Fauna and Flora (see D.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;

f) it should 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; and

h) a balanced diet should be provided to those species that eat a diverse range of foods.

D.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.

D.4 Procurement of eggs or birds

D.4.1 General

D.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).

D.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.

D.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.

D.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 and permit should have been granted before conducting field studies;

f) use of threatened or near 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 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;

NOTE All traps should be checked regularly, particularly in hot weather.

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 or surgical procedures should be carried out under sterile conditions, and appropriate anaesthesia and analgesia should be used.

D.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.

D.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.

D.7 Breeding and rearing birds from hatch

D.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.

D.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.

D.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.

n) Ideally, chicks should be reared in broods or groups of conspecifics and never in isolation, unless soundly justified for scientific reasons.

D.8 Diet for adult and juvenile birds

D.8.1 Birds vary in their digestive physiology and dietary requirements, and diets in captivity should reflect natural diets as closely as possible. Sufficient information about a particular species' nutritional requirements and digestive physiology should be obtained when formulating diet for a captive population.

D.8.2 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.

D.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.

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.

D.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.

D.11 Housing and husbandry

D.11.1 The environment

D.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.

D.11.1.2 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.

D.11.1.3 Birds should be inspected at least twice daily for abnormal behaviours and for any sickness.

D.11.1.4 A comprehensive health plan should be drawn up with a veterinarian before acquiring the birds.

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

D.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. For many species, outdoor aviary will be an appropriate option.

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

D.11.2.2 For indoor housing, large species, for example, ducks and turkeys should be housed between 15 °C to 20 °C, medium species, for example, quails and pigeons between 20 °C to 25 °C and small species, for example, most passerines between 25 °C to 30 °C. Within each species the optimal temperature range will vary with age class, with younger birds generally requiring higher temperatures. 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.

D.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.

D.11.2.4 In general the humidity birds experience in captivity should approximate that experienced naturally. For domestic species, relative humidity (see table D.1) should generally be maintained within the range of 50 % to 70 % for healthy adult birds.

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

1	2	3	4
Age Days	**Under lamp** °C	**Ambient room temperature** °C	**Relative humidity** %
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

D.11.3 Ventilation

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

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

D.11.4 Lighting

For indoor housing, lights should not be abruptly switched on or off, but dimmed or raised in gradual intensity. Dim "night lights" can be provided.

D.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.

D.12 Health

D.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 days) and habituation should be considered before wild birds are used for research purposes.

D.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.

D.13 Housing and housing

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

D.13.2 A good standard of health, well-being, 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.

D.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.

D.14 Feeding

D.14.1 General

D.14.1.1 Refer to D.8.1.

D.14.1.2 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.

D.14.1.3 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.

D.14.1.4 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.

D.14.1.5 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.

D.14.1.6 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*.

D.14.1.7 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 D.14.1.8) how to use feeders and water points to avoid starvation and dehydration.

D.14.1.8 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.

D.14.2 Watering

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

D.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).

D.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.

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

D.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.

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

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

D.16 Environmental enrichment

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

D.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.

D.17 Identification

D.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.

D.17.2 When chicks are ringed, regular monitoring is necessary to ensure that the rings do not restrict growth as the birds develop.

D.17.3 Electronic tagging should be done subcutaneously, or into the pectoral muscle, and never into the bone.

D.17.4 Use of multicoloured leg bands, or combinations thereof, can affect bird behaviour.

D.17.5 Highly invasive methods, such as toe clipping will cause suffering, and are not acceptable.

D.18 Record keeping

D.18.1 Records of all birds produced for, and used in, scientific studies should be kept.

D.18.2 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;

i) health and welfare records, including veterinary and laboratory reports;

j) deaths, post-mortems and findings; and

k) cage monitoring records.

D.19 Potential welfare issues

D.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.

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

D.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.

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

D.20 Routine scientific procedures

D.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, the brachial-vein will often be the most appropriate site.

b) The bird should be restrained carefully to prevent haematoma formation.

c) It should be ensured that bleeding has stopped before the bird is released.

d) Cardiac puncture should be performed under general anaesthesia.

NOTE This is a terminal procedure.

D.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.

d) It might be necessary to split the total dose between two sites. The recommended dose should not be exceeded.

D.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.

D.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.

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.

d) Abnormal behaviour should be regarded as an inability to cope with stress, the environment and procedures.

D.20.5 Anaesthesia

D.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.

D.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.

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.

f) Starvation can reduce hepatic detoxification of certain anaesthetic agents.

D.20.6 Anaesthetic agents

The following anaesthetic agents may be used:

a) Isofluorane – 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 D.20.6(b)). Recovery and induction is smooth.

d) Tiletamine and Zolazepam (Zoletil[1)] (5 mg/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 mg/kg 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.

D.20.7 Analgesics

The following analgesics may be used:

a) Buprenorphine – (0,02 mg/kg, im). Duration of effect on various species is uncertain.

1) Trade name for combination of Tiletamine and Zolazepam.

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).

D.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;

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;

e) a stimulating natural environment should be considered to enable released or re-homed birds to adjust rapidly; and

f) where possible, wild caught birds should be released at the site(s) of capture.

D.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.

e) Chilling and freezing are unacceptable.

D.23 Species requirements

D.23.1 Ducks and geese

D.23.1.1 General and special requirements

D.23.1.1.1 Ducks and geese are primarily adapted for locomotion and feeding in water. Their comfort behaviour is bathing and preening.

D.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.

D.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.

D.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.

D.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.

D.23.1.2 Water for bathing and swimming

All waterfowl should have some sort of pond (see table D.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 D.2 — Pond sixe 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.		

D.23.1.3 Social housing (see table D.2)

D.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.

D.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.

D.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.

D.23.1.4 Environmental enrichment

D.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.

D.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.

D.23.1.4.3 Nest sites and material

Sufficient nest sites and material should be ensured to prevent competition and aggression.

D.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.

D.23.1.4.5 Housing areas (see 10.7.2)

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.

D.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.

D.23.2 The domestic fowl (*Gallus gallus domesticus*)

D.23.2.1 Introduction

The domestic fowl has retained much of the biology and behaviour of the wild fowl from which it has descended.

D.23.2.2 Behaviour

D.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.

D.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.

D.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.

D.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.

D.23.2.3 Housing (see 10.7.2.3)

D.23.2.3.1 General

D.23.2.3.1.1 Ideally, domestic fowls should be housed (see table D.2) with access to outdoors with appropriate cover and refuge. Social stress is increased with inadequate space.

D.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).

D.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.

D.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 m^2 for birds less than 600 g body weight; and

b) 2 m^2 for birds over 600 g body weight.

D.23.2.3.2 Flooring and floor enclosures

D.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.

D.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.

D.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 D.23.1.4.6(d)).

D.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.

D.23.2.3.3 Perching

D.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.

D.23.2.3.3.2 Perches should be 3 cm to 4 cm in diameter, and round with a flattened top.

D.23.2.3.3.3 Perch heights vary with breed, age, size, and housing conditions. They should be fixed at 5 cm to10 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.

D.23.2.3.3.4 Each bird should be allowed 15 cm of perch space at each level.

D.23.2.3.4 Nesting boxes

D.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.

D.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).

D.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.

D.23.2.3.5 Feeding

D.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.

D.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.

D.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.

D.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.

D.23.2.3.6 Broilers

D.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.

D.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.

D.23.3 Quail (*Coturnix coturnix*)

D.23.3.1 General

D.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.

D.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.

D.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.

D.23.3.2 Temperature

D.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.

D.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.

D.23.3.3 Relative humidity

Quail can be maintained at a relatively wide range of humidity of 30 % to 80 %.

D.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.

D.23.3.5 Lighting

D.23.3.5.1 Lighting depends on the purpose for which the birds are being held.

D.23.3.5.2 Light or dark cycles vary from 14 h/ or 10 h to 8 h or 16 h (where there is a requirement to slow down growth rates).

D.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.

D.23.3.6 Housing (see 10.7.2.4)

D.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 D.23.3.6.3).

D.23.3.6.2 Indoor aviaries

D.23.3.6.2.1 Preference should 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.

D.23.3.6.2.2 Quail have a characteristic escape response that consists of sudden vertical flight movement. Pen heights should 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.

D.23.3.6.3 Cages (see table 4)

D.23.3.6.3.1 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.

D.23.3.6.3.2 Recommended minimum length of trough per bird is 4 cm.

D.23.3.7 Breeding systems

D.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.

D.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.

D.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.

D.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.

D.23.3.7.5 Female only groups can be satisfactorily maintained, but fighting is a serious problem in male groups.

D.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 days.

D.23.3.8 Incubation

D.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.

D.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 days. 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 days to 18 days. Hatching should be carried out in the hatching compartment of combined setter hatchers.

D.23.3.9 Environmental enrichment

D.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.

D.23.3.9.2 Females have a strong preference for ground cover (natural or artificial) especially at egg-laying and incubation periods.

D.23.3.10 Welfare issues

D.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.

D.23.3.10.2 Head injuries might be sustained by birds flying into the roof of the enclosure or pen.

D.23.3.10.3 The composition and quality of the diet is important to reduce skeletal and cardio-pulmonary problems in old caged birds.

D.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.

D.23.3.10.5 Battery housing systems are not acceptable for quail and should be justified if used.

D.23.3.10.6 Welfare problems increase with age in birds held in captivity, especially those held for more than a year.

D.23.4 Pigeons (Columbiformes)

D.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.

D.23.4.2 Social housing

D.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.

D.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.

D.23.4.3 Aviary housing

D.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.

D.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 10.7.2.5). Access to flight rooms with perches, for regular exercise, should be provided.

D.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.

D.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 should be avoided.

D.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).

D.23.4.7 Perches and nest boxes

D.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.

D.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.

D.23.4.7.3 Each bird should have 30 cm of perching space.

D.23.4.8 Diet and foraging

D.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.

D.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).

D.23.4.8.3 Cages should have covered food, grit and water hoppers.

D.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.

D.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.

D.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.

D.23.4.12 Welfare issues

Injuries might be sustained during capture, aggression or flight. Appropriate preventive measures and corrective action should be taken.

D.23.5 Finches and waxbills (fringillidae and estrildidae)

D.23.5.1 General

D.23.5.1.1 Finches and waxbills are delicate and require special care in captivity.

D.23.5.1.2 Finches and waxbills 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.

D.23.5.1.3 Finches and waxbills 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.

D.23.5.2 Social housing (see 10.7.2.6)

Fitting finches with brightly coloured leg bands can have a significant effect on social and reproductive behaviour.

D.23.5.3 Aviary housing

D.23.5.3.1 General

D.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.

D.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.

D.23.5.3.1.3 Outdoor access is beneficial wherever possible. Outdoor housing should provide adequate shelter and heating where necessary.

D.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.

D.23.5.3.3 Perches

D.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.

D.23.5.3.3.2 Wooden dowel rods or natural branches can be used.

D.23.5.3.3.3 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.

D.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.

D.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 should be provided. Appropriate nesting material, for example, dry grass should be provided. If aggression persists, divide the total number of birds into smaller groups and aviaries.

D.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.

D.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.

D.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) **Ectoparasites** 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 25 °C and 30 °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.

D.23.6 Ostriches

For the species-specific requirements for the handling and housing of ostriches, see SANS 994-1.

Annex E
(informative)

Care and management of cattle

E.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.

E.2 The environment

E.2.1 General (outdoors)

E.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.

E.2.1.2 If cattle are kept outdoors, they require proper shelter from the sun, wind, rain and other adverse weather conditions. They also require access to a well-drained area for rest and rumination. This area should be large enough to accommodate all cattle lying down at the same time.

E.2.2 Temperature (indoors)

E.2.2.1 Cattle housed indoors should generally be maintained at room temperatures between 8 °C and 26 °C.

E.2.2.2 In special cases, for example, when housing very young or recovering animals, higher room temperatures than those indicated (see E.2.2.1) might be required. Gradual acclimatization should be done before moving them outdoors after they have adapted to indoor conditions.

E.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.

E.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.

E.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.

E.2.4 Ventilation

E.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.

E.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.

E.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.

E.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.

E.2.5 Lighting

E.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.

E.2.5.2 Where natural lighting is not used, light and dark periods should be at least 6 h each per day.

E.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.

E.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.

E.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.

E.3 Animal care and health

E.3.1 General

E.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.

E.3.1.2 Cattle readily establish cohesive social structures. In so doing, they establish hierarchies and inter-individual relationships. When housed in barn-type housing, 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.

E.3.1.3 Cattle respond well to positive food reinforcement such as the provision of lucerne. Low stress handling can be achieved by competent, calm and confident personnel within an environment that is designed to assist such efforts.

E.3.2 Bedding material

E.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.

E.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.

E.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.

E.3.3 Feed and water

E.3.3.1 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.

E.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.

E.3.3.3 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 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.

E.3.3.4 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.

E.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.

E.3.3.6 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.

E.3.3.7 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.

E.3.4 Cleaning

E.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.

E.3.4.2 The facilities should be designed to support manure removal, cleaning and disinfection.

E.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.

E.3.4.4 Fly, tick and other pest populations should be regularly monitored and appropriate control measures should be applied when indicated.

E.3.5 Environmental enrichment

E.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.

E.3.5.2 The use of positive reinforcement in cattle, with food items such as lucerne, salt or barley as reward, is strongly encouraged.

E.3.6 Animal housing (see 10.7.3)

E.3.6.1 Cattle housing facilities should provide suitable access and restraining devices to allow animals to be inspected, caught or moved as necessary.

E.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.

E.3.6.3 If cattle are kept in a confined area over long periods, hoof trimming should be part of the herd management programme.

E.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.

E.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.

E.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.

E.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.

E.3.7 Breeding

E.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.

E.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.

E.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.

E.3.7.4 Detailed records should be kept of pedigrees as well as of fertility and rearing success.

E.3.8 Animal identification

E.3.8.1 General

E.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.

E.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.

E.3.8.2 Permanent marking

E.3.8.2.1 Ear-notching is not recommended for cattle.

E.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.

E.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.

E.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.

E.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.

E.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.

E.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.

E.3.9 Handling

E.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.

E.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.

E.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.

E.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.

E.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.

E.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.

E.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.

Annex F
(informative)

Care and management of Cephalopods

F.1 General

F.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.

F.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.

F.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.

F.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.

F.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.

F.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.

F.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.

F.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.

F.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.

F.1.10 Lifespan is generally short with the average being one year to two years for most species.

F.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 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.

F.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.

F.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.

F.2 Research

F.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.

F.2.2 Laboratory research into processes such as growth, reproduction, feeding and metabolism will require animals to be held for extended periods.

F.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.

F.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 F.6.2).

F.3 Capture methods and sources

F.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.

F.3.2 Cephalopods are usually sourced from the fishing industry as "secondary" or "by-catch". The condition of these animals should be assessed before being transported to laboratories.

F.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 two days to three days. Hand collection by scuba diving is also used in certain locations.

F.3.4 The use of bottom and pelagic trawls, seine nets, gill nets, lift nets and jigging lures is considered unacceptable.

F.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.

F.4 Handling and transport

F.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.

F.4.2 Small octopuses and sepioids may be placed in temporary containers partly filled with sea-water which should be changed regularly if the temperature, pH value and oxygen content alter significantly.

F.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.

F.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.

F.4.5 Copper is highly toxic to many marine invertebrates, especially to cephalopods. If copper has been used in a system in the past, it is necessary to build a new system for cephalopods, because it is extremely difficult to eliminate residual copper from a system.

F.5 Water quality

F.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.

F.5.2 The main parameters for the monitoring of water quality are temperature, salinity, pH, oxygen concentration, and levels of dissolved nitrogen (ammonia (NH_3), nitrite (NO_2), and nitrate (NO_3)). Water temperature should be as close to ambient as possible.

F.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 should 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.

F.5.4 The build-up of nitrogenous excretory waste products is a major problem for closed sea- water 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.

F.5.5 It is essential that water filtration is processed in the following order:

a) water leaves the animal holding tanks and then passes through a foam fractionator (protein skimmer), which strips dissolved organic compounds including ink,

b) the water then passes through a mechanical filter, removing particles down to 100 /lm,

c) it then passes through high-grade activated carbon, through a biologic filter where ammonia is broken down to less toxic forms by nitrifying bacteria (we generally use down-flow sub-gravel filters that have crushed oyster shell as a media), and

d) lastly through an ultraviolet (UV) sterilizer before returning to the animal holding tank.

F.5.6 System design should produce flow rates that allow the entire water volume of the culture system to pass through the filtration loop a minimum of two times per hour.

F.6 Space requirements

F.6.1 Certain sedentary species can be held in small enclosures, provided there is an adequate rate of exchange of water to maintain quality.

F.6.2 Octopods and sepioids quickly establish themselves in tanks of modest dimensions. Circular tanks are the best design.

F.6.3 Factors such as aggression and activity will determine tank size and numbers held.

F.6.4 Crowding may initiate aggressive behaviour, resulting in tank wall collisions and caudal mantle damage or cuttlebone fractures.

F.7 Housing and substrate

F.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.

F.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.

F.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.

F.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.

F.7.5 Any uneaten food should be removed as soon as possible from the tank.

F.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.

F.9 Feeding and food supplies

F.9.1 Feeding cephalopods are almost exclusively predatory carnivores and require a supply of live food. No artificial diet is currently available.

F.9.2 Octopuses capture and eat almost any crustacean of appropriate size, crabs, shrimps, prawns, squat lobsters, some varieties of gastropods and molluscs.

F.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.

F.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.

F.9.5 Feeder fish have the potential to introduce disease.

F.9.6 Cuttlefish have been trained to accept frozen fish or shrimp diet.

F.10 Growth

F.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.

F.10.2 Ratios for growth increment to food intake ranges from 40 % to 60 % for octopods and between 25 % and 40 % for squid.

F.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 %.

F.11 Lifespan

F.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.

F.11.2 After spawning, females die, almost without exception, within a couple of weeks. Males usually die after the first breeding season.

F.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.

F.11.4 Most cephalopods which reach full maturity in the laboratory will not spawn normally.

F.12 Damage and diseases

F.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.

F.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.

F.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.

F.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.

F.14 Cannibalism

F.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.

F.14.2 Animals that are sick or are dying are commonly eaten by others even when still alive.

F.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.

F.15 Culture and breeding

F.15.1 Culture and breeding under laboratory conditions is difficult and success will require specially favourable open seawater conditions or high-quality recirculation systems.

F.15.2 Handling of hatchlings and adequate appropriate food supplies are major concerns in laboratory and aquaria management.

F.16 Maturation

F.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.

F.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.

F.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.

F.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.

F.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.

F.18 Mating

F.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.

F.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.

F.18.3 Mating in cephalopods is always one-to-one although each individual can perform a series of matings with different partners.

F.19 Egg laying

F.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.

F.19.2 Sepioids lay small numbers (25 to 1 000) 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.

F.19.3 Octopods lay large eggs of length 2 mm to 15 mm and from 25 mm to 50 mm and up to 100,000 mm 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.

F.19.4 Culturing egg masses requires gentle circulation of clean aerated seawater. Direct agitation of the water from stirrers, skimmers, or aerators should be avoided and low-light levels should be maintained.

F.20 Hatchlings

F.20.1 Development time in the egg depends on the species and temperature. It generally ranges between 10 days and 100 days. Hatching embryos actively break out of the enclosing egg coats.

F.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.

F.20.3 Inflow and outflow pipes should be screened with fine mesh. Screened aeration inlets should be provided to protect hatchlings from air streams.

F.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).

F.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.

F.21 Handling

F.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.

F.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 should 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.

F.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.

F.21.4 Interaction with octopus after they have lain eggs or reached senescent should be discouraged.

F.22 Identity marking

F.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.

F.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.

F.23 Anaesthesia

F.23.1 Only anaesthesia by immersion is recommended.

F.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.

F.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.

F.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 should be returned to clean aerated sea-water. Recovery period is usually 5 min to 6 min.

F.23.5 Ambient temperature seawater should be used.

F.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).

F.23.7 Side effects that might be encountered are attempts to escape from the container and violent inking.

F.24 Surgical techniques

Octopods generally withstand surgical procedures carried out under anaesthesia well, provided procedures are carried out quickly and efficiently with minimal trauma.

F.25 Euthanasia

F.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.

F.25.2 Squid are killed by decapitation, which involves cutting between the head and the mantle.

Annex G
(informative)

Care and management of dogs and cats

G.1 General

G.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-parasiticides and endo-parasiticides, vaccine testing and development.

G.1.2 The international requirement for dogs and cats used for scientific purposes is that they shall be purpose-bred. The criteria for a study may however be such that purpose bred animals cannot be used. This would include natural infections, specified breeds of animals or specified age groups. Such animals may be rented or bought from consenting owners, but there are risks involved with obtaining animals from outside sources (see 10.3.4). Animals used for scientific and testing purposes are ideally obtained from reputable or registered sources. Under special circumstances, dogs can be sourced from consenting owners.

G.1.3 The choice of breed should be justified on animal ethics, the scientific purposes and on the breed that will produce the best scientific results. It should not be based simply on economics. The beagle is the most commonly preferred dog breed because of its relatively small size and placid temperament, ease of handling and housing, and the volume of scientific and genetic data on the breed.

G.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. Methods and mechanisms of restraint during a study should be humane and noted by the AEC.

G.1.5 Careful consideration should be given to balancing supply with demand within establishments, and to reduce surplus animals being produced in the breeding unit.

G.2 Behaviour

G.2.1 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.

G.2.2 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.

G.2.3 Animals, except those which are naturally solitary, should preferably be socially housed in stable groups of compatible individuals. In cases where single housing is allowed in accordance with A.5.1(b) the duration should be limited to the minimum period necessary and visual, auditory, olfactory or (or all of these) tactile contact should be maintained. The introduction or re-introduction of animals to established groups should be carefully monitored to avoid problems of incompatibility and disrupted social relationships.

G.2.4 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.

G.2.5 Animal care staff should be trained to recognize and correctly interpret animal signals and act accordingly using established husbandry routines to address signs of aggression, anxiety, fear and stress.

G.2.6 The need to retain animals showing marked abnormal or stereotypic behaviour should be questioned, since this constitutes an on-going welfare problem and is detrimental to scientific research data. Animals with abnormal or undesirable behaviour should be assessed by an animal behaviour expert to determine the best way forward in the interests of the animal.

G.3 Housing and husbandry

G.3.1 Location

G.3.1.1 The location and construction of the research animal facility should comply with institutional, local municipal and government regulations.

G.3.1.2 The facility should be situated away from sources of noise, pollution and traffic likely to cause stress or injury to the animals.

G.3.1.3 Areas that are prone to flooding and poor drainage should be avoided, especially where outdoor kennels and runs are planned.

G.3.1.4 Access to the site area should be controlled and should be for authorised personnel only.

G.3.2 Construction

G.3.2.1 All facilities should be properly planned and be of sound engineering design. Advice should be sought from a qualified animal behaviour expert when designing housing and exercise areas.

G.3.2.2 Materials used to construct pens and enclosures should be independent of the primary structure, so as to allow refurbishment and improvements with minimal disruption.

G.3.2.3 Kennels, pens and cages should be separated by either solid partitions or mesh wire dividers capable of preventing physical contact with neighbours.

NOTE 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 mm^2.

G.3.2.4 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.

G.4 Pens and cages

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 socialisation, including with humans.

G.5 Physical environment

G.5.1 Drainage

Kennels and cattery floors should be sloped to allow adequate drainage of waste and water.

G.5.2 Temperature

a) Dogs and cats have wide thermo neutral 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.

b) The optimum temperature range for adult dogs and cats is 20 °C ± 4 °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.

c) Newborn pups and kittens require a local (infra-red lamp or heating pad) environmental temperature range of 26 °C to 28 °C for at least the first 5 days to 10 days of life.

G.5.3 Relative humidity

Dogs and cats will tolerate a wide variation in relative humidity of between 30 % and 70 %.

G.5.4 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.

G.5.5 Lighting and light intensity

G.5.5.1 Lighting should be adequate to allow safe working and cleaning conditions, and efficient inspection of all animals.

G.5.5.2 Natural sunlight is the preferred means of lighting, but shaded areas are essential.

G.5.6 Noise

G.5.6.1 Background sounds, such as soft, preferably classic music, are considered beneficial.

G.5.6.2 Noise-producing equipment and machinery should be sited as far away from the animal housing as possible.

G.5.7 Bedding

G.5.7.1 Beds are recommended for sick animals, post-operative recovering animals, older animals (to reduce bed sores), and for periparturient and suckling mothers.

G.5.7.2 If, in a study, the use of bedding has a risk of contamination (for example, topical test items) or bedding may negatively affect the results of a study (for example, external parasites) bedding should not be used.

G.5.8 Security

G.5.8.1 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.

G.5.8.2 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.

G.6 Food and feeding

G.6.1 Sufficient palatable food of adequate nutritional value and highly digestible quality should be supplied daily to each animal.

G.6.2 Animals that are used to the laboratory environment should not be exposed to unnecessary stress and the type or brand of food that they have become used to should only be changed for accepted physiological reasons (for example, from puppy food to adult food and pregnancy or slactation).

G.6.3 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 days to 10 days.

G.6.4 Quality commercial foods are preferable. The manufacturer's instructions on the amount of food that is fed should be used as a guideline.

G.6.5 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 regimens (*ad libitum*, restricted, or in accordance with research study criteria).

G.6.6 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 and acted upon appropriately.

G.6.7 Animals should not be exercised directly after feeding.

G.6.8 For animals with poor appetite, providing a secluded and private place to eat will decrease stress and competition. Adding additional enticements might be necessary.

G.6.9 Spoiled, old, or mouldy food should be removed and disposed of. Residual uneaten food will attract vermin.

G.6.10 If prepared food is needed, it should be hygienically prepared in a separate diet kitchen area. Perishable foods should be refrigerated, and dry foods should be kept in a cool, vermin-free storage area for limited periods only.

G.6.11 All food bins should be covered with lids. Food bowls should be non-chewable, non-spill and easily cleaned and disinfected at least daily.

G.6.12 Group housed animals require one food bowl per animal.

G.6.13 Water should be clean, potable and unrestricted, and should be provided at more than one water point for group-housed animals. The quality of drinking water should be confirmed to be in compliance with SANS 241-1.

G.6.14 One litter tray per cat plus one extra tray should be provided.

G.7 Health

G.7.1 General

Dogs and cats require frequent human contact, and quality time should be spent with each animal.

G.7.2 Disease prevention

G.7.2.1 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 vaccination and treatment certificates.

G.7.2.2 The consultant veterinarian should provide advice on vaccination, treatment, and quarantine or isolation programmes.

G.7.2.3 Dogs and cats less than four months old, or animals suffering from an infectious disease, should not enter the facility, but if needed in exceptional circumstances they should be held in isolation.

G.7.3 Health checks and veterinary attention

G.7.3.1 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 who should take appropriate action to address any health issues.

G.7.3.2 A full-time, or consultant veterinarian, should be appointed to oversee the health and management requirements of the facility animals.

G.7.3.3 All unexpected deaths should be thoroughly investigated by the veterinarian and the necessary action taken.

G.7.4 Quarantine and isolation

G.7.4.1 Appropriate facilities and SOPs should be available for the isolation of newly introduced animals.

G.7.4.2 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 should be placed in quarantine.

G.7.4.3 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.

G.7.4.4 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.

G.7.5 Medication

G.7.5.1 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.

G.7.5.2 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.

G.7.5.3 Where authorized staff members administer medications, records should be kept of all administrations and filed for reference purposes.

G.7.5.4 Prophylactic medication should not be a substitute for good hygiene.

G.7.6 Exercise

G.7.6.1 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; allow muscular activity, and

c) have their behaviour, mobility and locomotion monitored by staff.

G.7.6.2 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. Do not confuse resting with hiding during stress conditions.

G.7.6.3 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.

G.7.6.4 Animal care staff should be on hand to supervise exercise periods, and to interact with animals.

G.7.7 Dogs exercise

G.7.7.1 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.

G.7.7.2 Very active dogs might require more exercise and in some cases exercise should be decreased based on the physical condition of the dog.

G.7.8 Cats exercise

G.7.8.1 Cats should have enough pen space (see 10.7.5.3) to allow them to stretch and move freely.

G.7.8.2 Cats should have access to an exercise area twice daily, at least for 1 h, if not adequately provided for in the housing pen.

G.8 Environmental enrichment

G.8.1 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. Care should be taken to provide adequate and appropriate stimulation to prevent undesired behaviour caused by a deprived environment.

G.8.2 Toys, scratch poles, and activity feeders are recommended as enrichment.

G.8.3 An animal behaviour expert should be consulted to develop a suitable enrichment programme.

G.9 Dogs environmental enrichment

G.9.1 Human contact is the main form of environmental enrichment. Personnel responsible for cleaning and feeding should support the actions of the personnel responsible for socializing.

G.9.2 In exercise runs the use of old tyres, wooden blocks or empty plastic bottles as well as safe structures to climb onto or crawl into can provide good stimulation. Do not provide edible products when animals are housed in groups.

G.9.3 In single cages, appropriate hard items should be provided for dogs to chew as this prevents boredom, gingivitis and periodontal disease.

G.10 Cats environmental enrichment

G.10.1 Pens and cages should contain

a) ample shelf room for resting (see 10.7.5.1 and 10.7.5.3.1),

b) objects suitable for climbing and claw care,

c) vertical structures for cats to scent-mark on,

d) toys and balls to play with, and

e) enclosed areas for safe retreat , for example, cardboard boxes, cat igloos.

G.10.2 Trees, bird baths and bird feeding trays in the vicinity of outside cat cages add to environmental enrichment.

G.11 Socialization, habituation and training

G.11.1 General

G.11.1.1 Animals should be given an acclimatization period of at least 7 days to 10 days after extended transport, and 3 days for relocation on site or between pens. At least 14 days should be allowed for acclimatization before the start of scientific studies.

G.11.1.2 Socially housed animals should be compatible. There are variations in temperament, and sympathetic husbandry practices should take this into account.

G.11.1.3 Advice on behavioural aspects should be sought from a qualified animal behaviour expert.

G.11.1.4 Positive interactions with humans should take place throughout the animal's life and the principles of positive reinforcement should be applied.

G.11.1.5 Dogs and cats can be trained to tolerate and accept various procedures during studies, including combing, brushing, clinical examination, rectal thermometer and collection of body fluids. The adaptation period before a study should be used for this purpose.

G.11.1.6 Frequent handling and vocal contact reduce the stress levels in animals and increase the ease with which they are handled in studies.

G.11.2 Dogs — Socialization, habituation and training

G.11.2.1 For dogs, runs should have sufficient length and a kennel/screen/hiding place for nervous or subordinate dogs to retreat.

G.11.2.2 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.

G.11.3 Cats — Socialization, habituation and training

Resting benches and feeding bowls in communal housing should be at different heights to support the social order of the animals in the cage.

G.12 Hygiene

G.12.1 Cleaning and disinfection

G.12.1.1 Animal housing and exercise runs should be kept clean so that the comfort and health of the animals can be maintained, and disease-controlled.

G.12.1.2 Faeces and residual food should be removed as quickly as possible and kennels and units cleaned at least once daily. Animals should only be returned to dry housing and should not be restricted to wet areas.

G.12.1.4 Before new animals are introduced or after any disease outbreak, animal housing should be thoroughly cleaned and disinfected, with a facility-approved disinfectant.

G.12.1.5 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.

G.12.2 Disposal of animals, biological and other waste

G.12.2.1 Waste disposal should be done in accordance with the requirements of the local municipal by-laws and the relevant national department (see foreword).

G.12.2.2 The person-in-charge should have a documented policy for dealing with unexpected or research-related deaths, or animal wastes. All staff should be made aware of these procedures, and the focus should be on biosafety.

G.12.3 Pest and vermin control

G.12.3.1 A facility should have SOP for the control of unwanted pests, flies and wild rodents.

G.12.3.2 Some pesticides and rodenticides are toxic to animals and should be used with care by designated trained staff. When handling toxic substances, protective gloves should be worn.

G.12.3.3 All animals should be excluded from treated areas until the poison programme is completed and no residual toxicity levels remain.

G.12.3.4 Care should be taken to ensure that study animals do not ingest the bait or vermin killed by the bait.

G.12.4 Emergency procedures (see 5.2.3.5.5(a)(3); 10.6.2.4.1; 10.6.2.4.3; 10.6.3.1.1 and A.2.7;)

G.12.4.1 An adequate plan and SOPs should be provided to cover all emergencies. All staff should be made aware of this plan and procedures involved.

G.12.4.2 Emergency contact numbers should be clearly displayed.

G.12.4.3 Emergency fire-fighting equipment should be placed at easily accessible areas, and emergency exits clearly marked.

G.12.4.4 Emergency power supply should be available.

G.13 Breeding management

G.13.1 Breeding systems

G.13.1.1 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.

G.13.1.2 Ultrasound scanning is the recommended method of pregnancy diagnosis. It allows earlier detection of pregnancy with an accurate prediction of the parturition date.

G.13.1.3 All breeding stock should be subjected to regular veterinary clinical health examinations.

G.13.1.4 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).

G.13.2 Selection of breeding stock

G.13.2.1 Behaviour assessments should be part of the selection process.

G.13.2.2 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.

G.13.2.3 Animals exhibiting appreciable levels of fear, long recovery times, abnormal behaviours, stereotypical behaviour, and reluctance to interact with handlers should not be considered for future breeding programmes.

NOTE Fear, abnormal behaviour etc. are not necessarily purely genetically influenced – management practices and experiential learning by the animals could also be at least partly responsible and should also be investigated as possible causes.

G.13.3 Minimizing losses and care of the new-born

Factors to be considered in minimizing losses of the new-born 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 new-born;

i) congenital defects;

j) early separation of the young from the mother; and

k) poor individual records on sires and dams.

G.14 Grouping

G.14.1 Single housing should be used in the following situations:

G.14.1.1 Long-term single housing and social isolation may lead to behavioural problems with dogs and cats but it may be necessary or even advisable to keep the dogs and cats in single housing.

G.14.1.2 The mixing and introduction of dogs and cats for short-term housing (up to a few weeks) should be discouraged owing to risk of disease transmission, social order aggression and anxiety.

G.14.1.3 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. However, these disadvantages can be mitigated by for example having observation systems in place and separate feeding of animals.

G.14.1.4 For aggressive animals as a last resort, ideally after, or in conjunction with an assessment by a behaviour expert. Additional daily human contact is necessary in such cases.

G.14.1.5 When group housing is incompatible with justifiable scientific objectives.

G.14.1.6 To prevent contamination with test substances, specifically topical products.

G.14.1.7 Established welfare reasons such as pregnancy, peri-parturition, injury, veterinary or disease isolation.

G.14.2 Single housing should adhere to the following requirements

Facilities for single housing should be designed and constructed in a way that the animals can see, hear and smell each other unless otherwise indicated (for example, aggression, anxiety).

G.14.3 Communal housing

G.14.3.1 Groups should be selected and monitored for social compatibility. Any aggression should be addressed immediately.

G.14.3.2 Grouping of animals during a study should only be attempted in established and compatible groups and only if the objective of a study requires group housing.

G.14.3.3 Because of the social order in groups, the competition between animals during feeding and the resultant increased stress levels, group housing during studies should be discouraged. Separate feeding can be considered.

G.14.3.4 Pens which house two compatible animals allow for social contact and facilitate the proper management of kennelled dogs provided that each dog's food and water intake plus health status can be easily observed and assessed.

G.14.3.5 Behavioural assessments should be done on new arrivals and used as a guide for group allocation and development purposes.

G.15 Balancing supply and demand

G.15.1 All possible measures should be taken to ensure that the supply of animals does not exceed demand, in order to minimize surplus animals.

G.15.2 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 rationalise the programme(s).

G.15.3 Regulatory authorities often require the inclusion of a single sex or specified weight range animals to support label claims for a single sex or defined body weight ranges. The necessity for such specifications should be questioned and justified.

G.15.4 Animals that display proven and untreatable undesirable behaviour or fear-related behaviour should be considered first for short-term terminal procedures, rather than for long-term use.

G.15.5 Relocation of aged ex-breeding animals to other establishments for use in scientific procedures is not acceptable (refer to G.23).

G.16 Transport

G.16.1 Attention should be given to relevant standards for transport, i.e to IATA Regulations, which will provide useful guidelines on transport and caging.

G.16.2 Animals should be transported in the shortest practicable time. Routes should be planned and provision made for emergencies.

G.16.3 Where animals have to be carried, their full body weight should be adequately supported. Dogs may be walked or lead-walked or transported in crates or trolleys. Cats should be carried in cages or baskets.

G.16.4 Any vehicle designed and used for animal transport should:

a) Protect the animal(s) from injury.

b) Have non-slip floors.

c) Provide easy and safe access for the operator.

d) Be well-ventilated and temperature-controlled. If not temperature controlled, it should be ventilated and travelling should be done during moderate environmental temperatures.

e) Protect against unauthorised release, or escape, of animals.

f) Be supplied with clean, secure cages.

g) Be supplied with clean, secure cages or carry baskets for small dogs or cats, and with separate compartments or partitions for larger dogs.

h) Be equipped to provide drinking water and food when animals are transported over long distances.

G.16.5 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.

G.17 Identification and records

G.17.1 It is good practice in any establishment to be able to uniquely identify each individual animal.

G.17.2 The method of identification should not be unnecessarily painful or cause mutilation or adverse reaction.

G.17.3 Non-invasive methods are recommended, especially for pre-weaned animals.

G.17.4 Minimally invasive methods are acceptable if an assessment indicates that permanent marking of individual animals is necessary.

G.17.5 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.

G.17.6 Invasive methods of identification include the following:

a) microchips (minimally invasive);

b) tattoos (on the ear, flank or lip); and

c) ear-notching.

NOTE Subcutaneous microchip implants (minimally invasive) are the recommended, most widely used and most satisfactory method of permanent identification. Tattooing and ear notching are not recommended, and if performed, local or general anaesthesia should be used.

G.18 Handling and restraint

G.18.1 One of the most important ways of minimising stress in animals in laboratory facilities is to ensure regular handling and attention and through fixed routines of cleaning and feeding procedures. All animals should experience adequate socialisation with humans during the primary socialisation period, combined with habituation and training studies. Where applicable, this process should continue throughout the animal's life.

G.18.2 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.

G.18.3 Stroking an animal is often beneficial. Direct eye contact and potentially threatening body postures should be avoided.

G.18.4 Temporary restraint can be stressful to animals, but habituation and training can reduce this. Duration of restraint should be as short as possible.

G.18.5 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.

G.18.6 Positive reinforcement methods should be used to train animals to tolerate restraint.

G.18.7 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.

G.19 Procedures

G.19.1 Administration of substances

G.19.1.1 It is a common research procedure to administer substances to animals.

G.19.1.2 The route used for administration of animal medicines will be the same as for the therapeutic use of the substance in animals.

G.19.1.3 The route for non-medicinal chemicals will be dictated by the regulatory guidelines. Administration of non-medicinal chemicals should be through the least aversive route possible and with the least potential for adverse effects over the whole procedure.

G.19.2 Removal of body fluids

G.19.2.1 When the collection of fluids, other than blood and urine, is necessary, the least invasive method(s) should be used.

G.19.2.2 Potential refinements for the removal of body fluids that involve repeated collections are vascular catheterisation for blood and urinary catheterisation for urine.

G.19.3 Metabolic cages

G.19.3.1 Metabolic cages should be scientifically constructed, their use soundly justified and authorised by an animal ethics committee, especially if alternative methods requiring less confinement and isolation are available.

G.19.3.2 The dimensions, design and construction of metabolic cages should be such as to minimize the impact on animal welfare.

G.19.3.3 Animals should be adapted to metabolic cages, including grid flooring, during procedural training.

G.19.4 Telemetry

G.19.4.1 Telemetry procedures and husbandry for animals fitted with telemetry devices should be refined to reduce any pain and distress.

G.19.4.2 Where jackets or collars are required, gradual habituation and monitoring for adverse effects is required.

G.19.5 Anaesthesia, analgesia and perioperative care

G.19.5.1 Where animals are expected to experience pain and distress during and after procedures, appropriate anaesthesia and analgesia should be administered.

G.19.5.2 Pre-anaesthetic evaluation of the animal(s) health status and condition should be carried out.

G.19.5.3 Appropriate anaesthetic and analgesia regimens should be researched and used.

G.19.5.4 Protocols for dealing with pain and chronic discomfort should be in place before a study begins.

G.19.5.5 Review of the perioperative care plan should be done regularly, and any improvements should be made, including recommendations for future protocols.

G.19.6 Recognising and monitoring adverse effects

G.19.6.1 Wherever possible, pain should be avoided, alleviated and prevented.

G.19.6.2 Before commencing a scientific procedure a list of likely or expected adverse clinical effects should be drawn up with mitigating treatments.

G.19.6.3 Retrospective assessments of clinical, surgical and perioperative procedures and treatments should be conducted.

G.19.6.4 Staff involved with perioperative care should be appropriately trained, able to recognize adverse reactions, and should take appropriate action to alleviate conditions.

G.19.6.5 Records of adverse reactions, treatments and any animal welfare related issues should be recorded.

G.19.7 Euthanasia

G.19.7.1 In accordance with the relevant national legislation (see foreword), 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 the animal alive, the veterinarian should forthwith destroy the animal or order the animal's destruction. A signed veterinary report and certificate might be required in such cases.

G.19.7.2 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.

G.19.7.3 Neuromuscular blocking agents should never be used alone to put animals to death.

G.19.7.4 It should be ensured that all members of personnel chosen to carry out euthanasia on animals are legally empowered, competent, willing, mentally prepared, and empathetic to carry out the process.

G.19.7.5 Euthanasia should always be carried out in an area separate from all other animals.

G.20 Long-term use

G.20.1 When maintaining animals for long periods in laboratory facilities, consideration should be given to the possible adverse effects of long-term housing, husbandry, and procedures.

G.20.2 Additional consideration should be given to care for the behavioural, social and physiological needs of these animals. Additional resources might be necessary.

G.20.3 The criteria for euthanasia should be considered, including the welfare cost of long-term confinement.

G.21 Adoption or re-homing

G.21.1 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.

G.21.2 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 keeping in mind that the animals are not house trained.

G.21.3 Adopted or re-homed animals will have to undergo a new socialisation, habituation and training programme in their new habitat. An animal behaviour expert's advice should be considered in this process.

G.22 Staff training

G.22.1 Staff should be aware of their responsibilities, should respect the animals and should be trained in the principles and application of low stress animal handling. Handling methods should be standardized. Record keeping of staff training is recommended.

G.22.2 Staff levels should be adequate for the size of the facility, and to manage all breeding colonies and husbandry practices.

G.22.3 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.

G.23 Animal welfare considerations

G.23.1 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 programme is essential to assist and inform staff of novel developments and practices in animal management, behaviour and welfare.

G.23.2 Suitable veterinary, hospitalisation, and isolation or quarantine facilities should be provided.

G.23.3 Adding complexity to the pen (by providing enrichment devices, toys, raised platforms, heating, runs, tactile contact, and view of conspecifics, etc.) is beneficial to animal welfare.

G.23.4 Chewing is an important behaviour and items should be provided which meet this need.

G.23.5 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.

G.23.6 Some animals 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.

G.23.7 Negative interactions, stressful handling, and restraint procedures should be minimized.

G.24 Records

G.24.1 Comprehensive records should be kept of each animal held in the facility and all animals should be identifiable.

G.24.2 Each animal should ideally have a documented permanent life case history.

G.25 Dogs and cats requirements

NOTE In addition to the preceding general statements and requirements the following species specific requirements should be considered.

G.25.1 Dogs

G.25.1.1 Dogs — Pen and cage size — Dogs (see table 8 to table 12)

G.25.1.1.1 Each animal should ideally have a documented permanent life case history.

G.25.1.1.2 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.

G.25.1.2 Dogs — Lighting and light intensity

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.

G.25.1.3 Dogs — Noise

G.25.1.3.1 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.

G.25.1.3.2 Elimination of vocal chords of dogs or use of electric shock bark collars are not acceptable.

G.25.1.4 Dogs — Security

Where dogs are housed, double fencing, an electric fence or a security barrier of at least 3 m high should be constructed to prevent animal escape or unauthorised entry.

G.25.1.5 Dogs — Care of the whelping bitch

G.25.1.5.1 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.

G.25.1.5.2 Bitches will seek solitude and privacy to whelp their young and need to be moved into the whelping kennel 2 days to 3 days before whelping, but within sight and sound of other pre-parturient and lactating bitches.

G.25.1.5.3 In the period 12 h to 24 h before whelping, the bitch's temperature may decrease from the normal 38,5 °C ± 0,5 °C to 36 °C ± 0,5 °C. The body temperature should be measured twice daily for several days before expected whelping in order to anticipate the whelping time.

G.25.1.5.4 Bitches should be provided with the opportunity to exercise for short periods from approximately 3 days postpartum.

G.25.2 Cats

G.25.2.1 Behaviour

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.

G.25.2.1.1 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.

G.25.2.1.2 Cats have a strong territorial nature and are therefore likely to become stressed if relocated.

G.25.2.1.3 Cats can develop complex social relationships, so a very important management aspect is the consideration of adequate space provision, the number and location of feeding, drinking, sleeping and elimination points in group-housed enclosures.

G.25.2.1.4 Cats do not always show obvious signs of stress. One of the most common signs of stress in cats is hiding, which is often misinterpreted as sleeping or resting. Recognizing stress in cats requires careful observation and an experienced caregiver.

G.25.2.2 Pen and cage size — Cats (see tables 12, 13 and 14)

G.25.2.3 Lighting and light intensity

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.

G.25.2.4 Noise

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 A.3.4). They should also be housed away from dogs.

G.25.2.5 Security

Catteries should have a double door system to prevent escape.

G.25.2.6 Food and feeding

G.25.2.6.1 Ideally, cats should be offered their daily food requirements, in divided portions, several times per day.

G.25.2.6.2 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 be placed at least 0,5 m from the litter trays to prevent contamination of food. Food and water bowls should preferably not be in the same location.

G.25.2.7 Litter trays

G.25.2.7.1 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 G.10.1(a)).

G.25.2.7.2 As 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.

G.25.3 Cats — Care of the periparturient queen and litter up to three weeks of age (see 10.7.5.3.3.2)

G.25.3.1 The pregnant queen should be housed singly only in late pregnancy, and preferably within the last week. They will seek isolation in a confined space for the act of parturition and for a period during the early suckling of their young. A quiet, private area should be provided for this purpose.

G.25.3.2 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.

G.25.4 Cats — Queen and litter from two weeks of age to weaning (See 10.7.5.3, tables 11 to 13 (inclusive) for cage sizes)

Cats should normally be housed socially. It is important that there is adequate social contact with other cats, and humans, during the primary socialization period (two weeks to eight weeks) with on-going social interaction up to 14 weeks. Post-weaned cats may be kept in the same sex groups.

Annex H
(informative)

Care and management of fish

H.1 General

H.1.1 Fish and aquaculture research focuses mainly on the areas of environmental or ecological pollution, conservation, protection of marine, estuarine and freshwater habitats, health and husbandry of food fishes, and molecular, genetic and toxicology studies.

H.1.2 Fish need to be maintained in controlled environments and emphasis should be placed on limitation of stress, humane handling and animal welfare aspects.

H.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.

H.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.

H.3 Capture and acquisition

H.3.1 General

H.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.

H.3.1.2 Collection of large numbers of animals from breeding populations, and unacceptable collection techniques and habitat destruction should be avoided.

H.3.1.3 Sampling equipment and strategies should be designed to minimize capture of non-target species.

H.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.

H.3.1.5 Sampling equipment (such as gill nets, seine nets and scoop nets) should be sterilized after each sampling trip to prevent the spread of pathogens from one water body to another (as is the case with the spread of the chytrid fungus affecting frog populations throughout most of the world.)

H.3.2 Representative samples

H.3.2.1 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.

H.3.2.1 Poor handling of large numbers of captured fish can result in high and unnecessary mortalities.

H.3.3 Collection of imperilled species

H.3.3.1 Imperilled species applies to those animals officially listed as threatened or Threatened and 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.

H.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.

H.3.3.3 Collection techniques, such as injurious or lethal ichthyocides, are not recommended.

H.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.

H.3.4 Wild fish and captive-bred fish

H.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 should 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.

H.3.4.2 Captive-bred fish are available from hatcheries and other laboratory supply houses, aquaria, and hobbyists.

H.3.4.3 Applicable animal welfare laws should be considered.

H.3.5 Killed and museum specimens

H.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.

H.3.5.2 Each animal collected should serve as many types of study as possible to reduce the total numbers collected to a minimum.

H.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.

H.3.5.4 Fish should be put to death by recognized euthanasia methods before immersion in formalin.

H.3.6 Acquisition of hatchery fish

H.3.6.1 Fish should come from hatcheries with defined and acceptable health status, and preferably known genetic history.

H.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.

H.4 Transport

H.4.1 Contingency plans should be drawn up for any vehicle breakdowns during transport.

H.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.

H.4.3 Fish may be taken off food for two days to three days before being transported. They will then have voided their digestive tract contents and will not excessively foul transport water.

H.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 plastic 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 plastic bags, and should be transported in more durable containers.

H.4.5 The packing method in H.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.

H.5 Quarantine and acclimatization

H.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 should be approved by the IBSC to ensure continued biosafety standards.

H.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.

H.5.3 Personnel working in the quarantine facility should be adequately trained in quarantine procedures and disease recognition.

H.5.4 A quarantine period of 30 days 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 hand basins, paper towel dispenser and approved detergent or surgiscrub dispensers.

H.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 comprehensive tank records should be kept. Separation distance between tanks should be such as to prevent splashing of water from one tank to another.

H.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.

H.5.7 Fish from transport tanks should be given enough time to acclimatize. Water temperature in the quarantine tank or transport tank should be adjusted until they are within one degree of each other before fish are removed from transport tank and placed in the quarantine tank. No water should be swapped as you will be introducing sedatives into the quarantine system.

H.5.8 On entering the quarantine facility, fish should be inspected for any abnormalities and external lesions. Appropriate specimens should be taken. The lesions may be routinely treated with approved broad-spectrum antibiotics, antiparasiticides, and antifungal agents.

H.5.9 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.

H.5.10 Dead fish should be removed immediately and post-mortem or laboratory testing (or both) should be carried out.

H.5.11 All designated cleaning and other equipment should remain in the quarantine area.

H.5.12 Lighting provision should be adequate for inspection purposes. Dimmer systems may be incorporated.

H.5.13 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.

H.5.14 The coving in the holding room should be at least 150 mm high to contain any accidental water spills (for example, from tank ruptures).

H.5.15 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.

H.5.16 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 relevant national department (see foreword).

H.6 Quality assurance (QA) 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.

H.7 Animal welfare considerations

H.7.1 General

H.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.

H.7.1.2 Regulatory requirements covered by the relevant national departments (see foreword) should be observed. There should be as little as possible or no disturbance of natural habitats. The use of experienced personnel is essential.

H.7.1.3 Appropriate attention should be given to study design and procedures whilst ensuring the humane treatment of study subjects.

H.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.

H.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.

H.7.2 Pain and distress

H.7.2.1 Researchers should take great care to avoid inducing stress and pain in fish research subjects, especially on a prolonged basis.

H.7.2.2 In fish, any deviations from normal homeostasis, will result in stress.

H.7.2.3 Appropriate use of anaesthetics and analgesics in procedures that can cause pain is essential.

H.8 Aquatic facilities and housing

H.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.

H.8.2 Types of systems (flow through, recirculation and static)

H.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.

H.8.2.2 Correct water management is critical for the well-being 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.

H.8.3 Effective environmental monitoring and control of tanks

H.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.

H.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.

H.8.4 Water quality and management

H.8.4.1 General

H.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).

H.8.4.1.2 All monitoring equipment should be regularly serviced and calibrated.

H.8.4.1.3 Detailed records should be kept of all maintenance and repairs for retrospective analysis.

H.8.4.2 Temperature

H.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.

H.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.

H.8.4.3 Oxygen and super-saturation

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 should be avoided by appropriate regulating mechanisms.

H.8.4.4 pH value

H.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.

H.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.

H.8.4.5 Salinity, alkalinity and hardness

H.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.

H.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 should be monitored.

H.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.

H.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.

H.8.4.6 Nitrogenous compounds and toxic agents

H.8.4.6.1 Nitrogen is present in water as gas, nitrates, nitrites and ammonia.

H.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.

H.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.

H.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.

H.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.

H.8.5 Engineering, design and materials

The correct materials (for example, concrete, plastics, fibre, glass and glues) should 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.

H.8.6 Mechanical and electrical requirements

H.8.6.1 All electrical systems should be professionally installed and should comply with the relevant national standards.

H.8.6.2 Extension cords and system(s) overloading should be avoided.

H.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.

H.8.6.4 Machinery that produces noise and vibration should be isolated from the areas housing fish (see A.3.4).

H.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.

H.8.7 Lighting

H.8.7.1 Both photoperiod and light intensity, and the variations for each species, are important.

H.8.7.2 Most species do well at a 12 h or 12 h light or dark cycle, although some tropical fish prefer a 10 h or 14 h light or dark cycle.

H.8.7.3 Fluorescent lighting is most commonly used. Full spectrum lighting may be used over tanks.

H.9 Husbandry and breeding

H.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.

H.9.2 Density and carrying capacity

Each species should be housed at a density that optimizes the well-being 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.

H.9.3 Food, feeding and nutrition

H.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.

H.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.

H.9.3.3 Fish food should only be purchased from sources approved by the person-in-charge, and in accordance with the standards required.

H.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.

H.9.4 Broodstock and breeding

H.9.4.1 Holding systems and environmental conditions should be appropriate for the species being held.

H.9.4.2 Attention should be given to environmental cues for the maintenance, stimulation, or manipulation of endogenous reproductive rhythms.

H.9.5 Environmental enrichment

Environmental enrichment should be provided (see 10.7.6). Although some species require plants, refuge areas or gravel to exhibit natural behaviour, fish should be held in clean aquaria (tanks) with no gravel or plants as they make it more difficult to keep the tanks clean and to observe any abnormalities that may be present. If gravel and plants are placed in the tanks care should be taken to make sure the gravel is inert and that the plants are not carrying any pathogens. Plants should be indigenous as far as possible to prevent the spread of alien invasive plants into our waterways.

H.10 Health and disease control

H.10.1 Fish health programme

H.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.

H.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.

H.10.1.3 Dead fish should be incinerated.

H.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 of all treatments should be kept.

H.10.2 Injuries and handling

H.10.2.1 Fishes should be fasted before handling or manipulation.

H.10.2.2 Personnel that handle fish should be trained and be experienced to reduce handling injuries.

H.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.

H.10.2.4 Fish should not be kept in the open air for more than 30 s.

H.10.3 Vermin control

Surveillance should be maintained for the presence of unwanted vermin, and a control programme should be undertaken if required.

H.11 Laboratory studies using fish

H.11.1 Fish should not be held indefinitely without the AEC approved protocol.

H.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.

H.11.3 Key personnel should be listed.

H.12 Study procedures

H.12.1 Statistical design

H.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.

H.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 or senior researcher should be consulted to develop study designs that have the appropriate statistical power to accomplish sound objectives.

H.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.

H.12.3 Surgery

H.12.3.1 Surgery should be performed by personnel with appropriate training and expertise.

H.12.3.2 Surgical sites should be prepared in a sterile manner which also minimizes tissue damage and contamination.

H.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.

H.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.

H.12.3.5 Any incisions should avoid the lateral line and should follow a longitudinal axis.

H.12.3.6 Suture materials should be strong, inert, non-hygroscopic and be placed with atraumatic needles.

H.12.4 Administration of compounds and devices

H.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).

H.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.

H.12.4.3 Intraperitoneal injections should avoid penetrating the abdominal viscera.

H.12.4.4 Implanted materials should be biocompatible and aseptic, and be implanted using sterile techniques.

H.12.4.5 Care should be taken to introduce the suture needle in spaces between the scales.

H.12.5 Marking and tagging

H.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.

H.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.

H.12.5.3 Release of fish back into the wild should comply with relevant regulations (see foreword). 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.

H.12.6 Collection of body fluids and tissue

H.12.6.1 Results obtained from careful collection and examination of blood and tissue specimens are often critically important for research results. Sterility under field conditions is not always possible, and procedures should be designed to minimize contamination.

H.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.

H.12.6.3 Blood is sampled 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.

H.12.7 Endpoints and monitoring

H.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 the AEC approved protocol.

H.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.

H.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.

H.12.7.4 The frequency of monitoring should allow for the timely removal of fish, before severe morbidity occurs.

H.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.

H.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.

H.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.

H.12.11 Genetically modified fish

Genetically modified fish (transgenic fish) should not be permitted to enter the food chain.

H.13 Holding and disposition of study fish

H.1.3.1 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.

H.1.3.2 Work with many species is regulated by the provisions of CITES.

NOTE 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.

H.14 Dangerous aquatic fish and safety considerations

H.14.1 It is important to note the human safety aspects when working with fish of unknown origin and health status. The risk of zoönoses 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.

H.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.

H.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.

H.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.

H.15 Anaesthetics and analgesics

H.15.1 General

H.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.

H.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.

H.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.

H.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 should be provided in the anaesthetic solution as hypoxia is a potential side effect.

b) **Benzocaine and benzocaine hydrochloride** is a highly insoluble powder that should 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.

e) **2 phenoxyethanol** has been used very effectively in fish transport as a sedative at 100 mL/1 000 L. It has been proven effective in many studies and is not harmful to the person administering them.

f) **Clove oil** can be used as an anaesthetic at concentrations of 40 mg/L. It has been proven effective in many studies and is not harmful to the person administering them.

H.15.2 Stages of anaesthesia in fish

H.15.2.1 **Stage 1** – erratic swimming, excitement, some loss of equilibrium, disorientation, increased respiration, some loss of tactile response and reduced activity.

H.15.2.2 **Stage 2** – loss of equilibrium, slow swimming movements with loss of direction, and decreased respiration.

H.15.2.3 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.

H.15.2.4 Stage 4 – spasmodic over distension of opercules and cardiac failure.

H.16 Euthanasia

Where euthanasia in fish is required, the following process should be used wherever possible:

a) anaesthesia to loss of equilibrium; followed by

b) physical or chemical method to cause brain death.

NOTE MS-222 is recommended at 500 mg/L followed by an acceptable method to ensure brain death.

c) double pithing.

NOTE In parasitological work and eco-toxicological work the addition of the anaesthetic can often affect the results as you are adding a variable into the mix. Severing the spinal cord and then destroying the spine negates this problem.

Annex I
(informative)

Care and management of horses

I.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.

I.2 The environment

I.2.1 General (outdoors)

I.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.

I.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 as well as additional space to allow for an animal to retreat in case of a threatening incident.

I.2.2 Temperature (indoors)

I.2.2.1 Horses housed indoors should generally be maintained at room temperatures between 16 °C and to 22 °C.

I.2.2.2 In special cases, for example, when housing very young or recovering animals, higher room temperatures than those indicated (see I.2.2.1) might be required. Gradual acclimatization should be done before moving them outdoors after they have adapted to indoor conditions.

I.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.

I.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.

I.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.

I.2.4 Ventilation

I.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.

I.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.

I.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.

I.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.

I.2.5 Lighting

I.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.

I.2.5.2 Where natural lighting is not used, light and dark periods should be at least 6 h each per day.

I.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.

I.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.

I.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.

I.3 Animal care and health

I.3.1 General

I.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.

I.3.1.2 When housed in barn-type housing, 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.

I.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.

I.3.1.4 All horses entering the holding facility should preferably have pharyngeal swabs taken to check for Strangles,

I.3.2 Bedding material

I.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. Large wood shavings are preferable in horses that have airway problems. The shaving should not be treated with any chemicals. A minimum average layer thickness of at least 15 cm of bedding material is recommended.

I.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.

I.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.

I.3.3 **Food and water** (see table I.1)

I.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. Water intake should be monitored daily. Horses should be drinking at least 40 ml/kg to 60 mL/kg/24h. Automatic water suppliers should be checked on a daily basis.

I.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 with sufficient spaces between them 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. Feeding and water troughs should as far as possible not be erected in corners as to allow adequate space for a horse to retreat if approached by a threatening horse (i.e. prevent it from being cornered and “bullied” without a chance to escape).

Table I.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

I.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. Camp inspections should be performed on a regular basis to remove any objects or materials that can injure the horses.

I.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.

I.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.

I.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.

I.3.4 Cleaning

I.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. Animal attendants should clean horse holding areas safely and with little stress to the horse. Cleaning staff should be trained in biosecurity.

I.3.4.2 Strict wash down periods for biosecurity should be adhered to. Stables should be emptied, washed with a high pressure hose and then fogged with a registered veterinary disinfectant before and after use. Bacterial swabs of stables should be taken after disinfection to monitor efficiency of cleaning

I.3.4.3 The facilities should be designed to support manure removal, cleaning, disinfection and drainage of urine away (indoor facilities).

I.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.

I.3.4.5 Internal parasites, external parasites (flies and ticks) and other pest populations should be regularly monitored and appropriate control measures be applied when indicated.

I.3.4.6 Bedding should be replaced regularly. Faeces and soiled bedding (urine) should be removed on a daily basis if horses are kept indoors.

I.3.5 Environmental enrichment

I.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 mud baths, or rubbing against poles and trees.

I.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.

I.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.

I.3.6 Animal housing (see table 15)

I.3.6.1 Equine housing facilities should provide suitable access and restraining devices to allow animals to be inspected, caught or moved as necessary. Provision should be made for an isolation or quarantine facility.

I.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.

I.3.6.3 If horses are maintained over long periods, hoof trimming and teeth floating (or checking teeth) should be part of the animal management programme.

I.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.

I.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.

I.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.

I.3.7 Breeding

I.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.

I.3.7.2 New born foals require adequate nutrition and a high level of hygiene. Mothers and their offspring should be disturbed as little as possible.

I.3.7.3 Detailed records should be kept of pedigrees as well as of fertility and rearing success.

I.3.8 Animal identification

I.3.8.1 General

I.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.

I.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 abcessation is minimal.

I.3.8.2 Permanent marking

I.3.8.2.1 The physical description of permanent signs such as colour, markings, breed and position of hair whorls, feathering and scars, is one of the most common identification systems for horses.

I.3.8.2.2 Ear-notching is not recommended for horses.

I.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.

I.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.

I.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.

I.3.8.2.6 Freeze-branding might be required under some circumstances (hot branding or hot ironing should be avoided as it is more painful). Freeze branding should be performed by experienced personnel with adequate equipment and appropriate sedation and analgesia.

I.3.8.3 Semi-permanent marking

I.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.

I.3.8.3.2 Hoof branding might also aid identification.

I.3.8.4 Temporary marking

I.3.8.4.1 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.

I.3.8.4.2 Placing different coloured ribbons braided into the mane can stay on for extended periods of time.

I.3.9 Handling

I.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.

I.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.

I.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.

I.3.9.4 Care takers or animal attendants should be taught to recognise an ill horse or a horse in pain and to report back their findings to the person in charge of the horses. Daily inspections should be performed by the caretaker and any abnormal clinical signs should be reported to the appropriate person who will decide if a veterinarian should be consulted.

I.3.9.5 Horses should be groomed several times a week, particularly when they are shedding, and the hooves should l be cleaned daily.

I.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. Records of corrective actions that were taken including but not restricted to veterinary treatments, hoof trimming, teeth floating etc. should also be kept.

I.4 Disease prevention

I.4.1 Horses should be regularly vaccinated against appropriate diseases taking into account the locality and risk of the disease. A veterinarian should be consulted in this matter. All horses should be at least once a year subjected to a veterinary clinical examination.

I.4.2 Sick horses should be isolated from the other horses in an isolation facility or quarantine facility.

I.5 Transporting

Horses should be transported in a humane way taking into account the distance to be travelled, ambient temperature and manner of transport. For long distances: resting periods and water or food should be provided for.

I.6 Re-homing and euthanasia

I.6.1 In accordance with the relevant national legislation (see foreword), 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 the animal alive, the veterinarian should forthwith humanely destroy the animal or order its destruction.

I.6.2 The possibility of adoption or re-homing, as an alternative to euthanasia, should be considered in conjunction with the animal ethics committee. Only healthy animals, and those not suffering any adverse effects from their experiences in the laboratory, should be considered.

I.6.3 The adoption or re-homing process should consider all the issues relating to animal welfare, the capabilities of the new owner, the requirements of the relevant regulatory authorities (see foreword) and the new environmental conditions.

Annex J
(informative)

Care and management of non-human primates (baboons, vervet and rhesus monkeys)

J.1 General

The information supplied in this annex is confined to baboons (*Papio ursinus*), vervet monkeys (*Chlorocebus aethiops* previously *Cercopithecus aethiops*), and rhesus monkeys *(Macaca mulatta),* 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.

J.2 International guidelines

International guidelines are not always comparable and may vary between countries. For example, the USA minimum cage dimensions for primates are smaller than those of the European standards. (Reinhardt *et al*. 1996). These international primate housing standards have been adopted for African primates.

J.3 General guidelines

Primate caging or housing systems should meet the physical, physiological, psychological and behavioural needs of captive primates within the confines of management and research requirements. 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, should 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.

J.4 Special considerations regarding species differences

Some research conducted on vervet monkeys in an indoor facility (Seier *et al*. 2004) 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 and Sargi, 1995, Ankel-Simons 2000).

f) Rhesus monkeys are seasonal breeders, while vervets and baboons are not.

g) Rhesus monkeys display aggressive behaviour (i.e. gang attacks) in harem breeding systems. The compatibility of individuals in communal housing is very important, and has to be carefully determined and monitored.

h) Size and weight; and

i) Social structure and troop size.

NOTE Stereotypical and other abnormal behaviours can develop in all these species. (Kessel and Brent 2001).

J.5 General considerations

J.5.1 Primates should only be housed in single cages of minimum dimensions for the absolute shortest period of time required to successfully conclude the study.

J.5.2 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 to facilitate the recovery period by preventing excessive movement, transport or quarantine. Professional judgement should be applied in these cases.

J.5.3 Facilities are strongly encouraged to exceed the minimum cage sizes.

J.6 The environment

J.6.1 General

J.6.1.1 Although baboons, vervet and rhesus 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.

J.6.1.2 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 and prevailing winds.

J.6.1.3 Low-ranking or otherwise weaker members of a group might be ousted from shelters by dominant troop members. Care should be taken to provide enough shelters (for example, multileveled perches), for such members of a group, considering social composition and dynamics.

J.6.2 Indoor facilities

J.6.2.1 Temperature

J.6.2.1.1 A good temperature range for baboons, vervet and rhesus monkeys is 22 °C to 26 °C, but size, age and condition of the animals have to be considered.

J.6.2.1.2 Monitoring and recording of temperatures, and other considerations, is as recommended in the relevant clause for rodents (see M.2.2).

J.6.2.2 Relative humidity

A humidity range of 30 % to 70 % is considered acceptable for most Old World species.

J.6.2.3 Ventilation

J.6.2.3.1 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.6.2.3.2 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.6.2.3.3 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.

J.6.2.3.4 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.6.2.4 Lighting

J.6.2.4.1 The lighting should provide good visibility and enable complete routine inspection of animals and animal rooms at all levels (lux levels of 200 to 500 are often recommended for commonly kept species).

J.6.2.4.2 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 D3 to prevent deficiency of this micronutrient.

J.6.2.4.3 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.

J.6.2.4.4 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. A behavioural warning sign would be animals huddling and sleeping during the day. Therefore, automatic timers should control light cycles in all animal rooms. Timer function should be monitored by an alarm system. A daily cycle of 12 h dark:12 h light is usual.

J.6.2.5 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. Particular care should be taken during building renovations and repairs.

J.6.2.6 Vibration

J.6.2.6.1 Care should be taken not to site animal rooms near a constant or intermittent source of vibration.

J.6.2.6.2 Vibration stability will be of greater concern if the research animal facility is located on the upper levels of a building rather than at ground level because of structural considerations.

J.6.2.7 HVAC (heating, ventilation and air conditioning systems)

Indoor facilities that rely on HVAC should have standby systems to maintain air- conditioning and lightning.

J.7 Animal care and health

J.7.1 General

Proper animal care includes providing for the physical and behavioural well-being of captive primates and preventing and controlling disease and injury.

J.7.2 Bedding/substrate

Although bedding/substrate absorbs urine, in primate care it is more frequently used to enable foraging. The material, such as wood shavings/corncob (indoor) or stones (outdoor) 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), this is quite rare, and the benefits outweigh the disadvantages.

J.7.3 Food and water

J.7.3.1 Automated supply of uncontaminated potable water should be provided to animals in sufficient quantity. In communal housing it is important to ensure that there are sufficient feeding and watering stations to avoid undue competition. Water nipples and pipes from automatic water systems should be checked and flushed regularly.

J.7.3.2 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.

J.7.3.3 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, which provides all required nutrients For detailed information, consult "*Nutrient Requirements of Nonhuman Primates*". 2nd Revised Edition, 2003, Nutritional Research Council,

J.7.3.4 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.

J.7.3.5 Standardized pelletted 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). Therefore, the easiest and most scientifically sound way of providing food diversity is by supplementing the diet with low-sugar fruit and vegetables. It is important to note that the food type plays an important role in dental health.

J.7.4 Cleaning

J.7.4.1 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.7.4.2 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.

J.7.4.3 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 approximately once a week depending on the thickness of the bedding, at which time the surfaces should also be sanitized.

J.7.4.4 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.

J.7.5 Environmental enrichment

J.7.5.1 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.

J.7.5.2 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.

J.7.5.3 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.

J.7.5.4 The environmental enrichment plan should address the following broad issues:

a) social contact;

b) foraging;

c) manipulanda (for example, food puzzles, water);

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).

J.7.6 Animal housing (see 10.7.8)

J.7.6.1 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. 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 should 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.

J.7.6.2 There should be space to separate injured animals from the social group. This can sometimes be achieved by placing a secondary cage within the primary one. It may alleviate aggression and maintain social structure.

J.7.6.3 The following morphological parameters should be taken into consideration in the design of both cages and perches.

Table J.1 — Species maximum morphological parameters

1	2	3	4
Species	**Weight**[a] kg	**Crown rump length(sitting height)** Cm	**Crown heel length(standing height)** cm
Baboons	30-35 up to 44	80	About 125
Vervet	5,50	49	76
Rhesus	11	48	87

[a] The body weights have consequences for the height of the sitting perch and cage size; therefore, only the dimensions of males are important.

J.7.6.4 The following points should also be taken into account when deciding on cage sizes for vervet and rhesus monkeys (see tables 16 and 17):

a) the cage should be high enough to enable installing a resting perch, so that a fully-grown male monkey can sit comfortably under as well as on top of the resting perch; and

b) If the animals are kept in cages that comply with the minimum dimensions only for extended periods or that are less than 1,8 m, 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.

J.7.7 Breeding

J.7.7.1 Baboons, rhesus 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 J.7.5). 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, and for some species.

J.7.7.2 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. Weaning should always take place in peer groups.

J.7.8 Animal identification

J.7.8.1 General

J.7.8.1.1 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.7.8.1.2 In choosing an acceptable marking technique, the researcher should consider the nature and duration of restraint, whether or not pain, if inflicted, is momentary or prolonged, and whether the risks of infection and abcessation is minimal.

J.7.8.2 Permanent marking

J.7.8.2.1 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 large gauge of the implanting needle, the implantation of microchips should always be performed under general anaesthesia in sterile conditions.

J.7.8.2.2 Tattooing on the inner thigh or chest is another less expensive way of positively identifying individuals. A tattoo should be applied under anaesthesia.

J.7.8.3 Semi-permanent marking

A patch of fur or patterns may be shaved, clipped or cut with a pair of scissors.

J.7.8.4 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.

J.7.9 Handling

J.7.9.1 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.

J.7.9.2 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.

J.7.9.3 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 large species.

J.7.9.4 Physical handling should only be done by highly skilled people.

J.7.10 Health monitoring and record keeping

J.7.10.1 Permanent electronic or manual records should be kept for every individual animal. These should contain identification number or name, date of birth (if available), identification of parents, general health status, reproductive history and experimental use. All records should be maintained for a minimum of five years.

J.7.10.2 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.

Annex K
(informative)

Care and management of pigs

K.1 General

K.1.1 Pigs are important models in biomedical research, and their numbers in research are increasing, as they continue to replace other non-rodent species. Their anatomical and physiological properties have many similarities to those of the human, but their differences should also be recognised and appreciated.

K.1.2 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's well-being is largely determined by the amount and quality of care and the operator understands of the animals.

K.1.3 Pigs are very sensitive and intelligent animals that require special attention and care as specified in SANS 1478, to ensure their physical and behavioural well-being.

K.1.4 The five freedoms of pigs should be ensured at all times:

a) freedom from hunger and thirst;

b) freedom from pain, injury and disease;

c) freedom from disease;

d) freedom from discomfort; and

e) freedom to express normal behaviour.

K.2 The environment

K.2.1 General

K.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.

K.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 comfortably at the same time.

K.2.2 Temperature

K.2.2.1 Pigs are sensitive to temperature fluctuations and extreme temperatures. Swine are best housed at the thermo-neutral temperature; at which relative humidity appears to be unimportant as energy conversion is not influenced by humidity at the thermo-neutral temperature. The following are recommended:

a) suckling 0 weeks to 5 weeks 30°C;

b) juveniles 6 weeks to 8 weeks 24 °C;

c) young 14 weeks to 16 weeks 20 °C; and

d) adults 18 °C.

NOTE If on raised grid floors, due to increased heat loss, the temp should not go below 20 °C. Humidity can then be maintained at 50 % to 70 %.

K.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.

K.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.

K.2.2.4 In special cases, for example, when housing very young or recovering animals, higher room temperatures than those indicated in K.2.2.1 might be required. Gradual acclimatization to outdoor conditions needs to be made before moving them outdoors.

K.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 micro-environment should also be monitored.

K.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.

K.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.

K.2.4 Ventilation

K.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.

K.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.

K.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 small livestock under conventional housing conditions.

K.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.

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. Light tubes, which imitate the spectrum of sunlight, are commercially available and their use is recommended.

K.2.5.2 Where natural lighting is not used, light and dark periods should be at least 6 h each per day.

K.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.

K.2.6 Noise

K.2.6.1 Sudden irregular noises create more disturbances in pigs than continuous or predictable sounds. Pigs are particularly sensitive to sudden, loud noises, and these should be avoided. Housing personnel should announce themselves in a uniform manner to avoid startling the pigs.

K.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.

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 cage wash, 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 research 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

K.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.

K.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.

K.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.

K.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.

K.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.

K.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 should be removed from the pen and treated.

K.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.

K.3.2 Bedding material

K.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.

K.3.2.2 Pigs should be given the opportunity to create their own toilet, sleeping and feeding areas.

K.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.

K.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.

K.3.3 Food and water

K.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.

K.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.

K.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.

K.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.

K.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.

K.3.4 Cleaning

K.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.

K.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 K.3.2.1).

K.3.4.3 Cleaning and removal of excrement should be done on a daily basis. Removal of soiled bedding is essential. If housed on grid floors, the grid floors should be checked on a daily basis. Pens should be washed weekly and sanitized thoroughly on a monthly basis.

K.3.4.4 Fly, tick and other pest populations should be regularly monitored and appropriate control measures be applied when indicated.

K.3.5 Environmental enrichment

K.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.

K.3.5.2 The widely used practice of feeding pigs twice daily is unsatisfactory from both behavioural and physiological points of view. Pigs should either be fed *ad libitum* or food should 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.

K.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).

K.3.6 Animal housing (see table 18)

K.3.6.1 Pig housing facilities should provide suitable access and restraining devices to allow animals to be inspected, caught or moved as necessary.

K.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.

K.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.

K.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 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.

K.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.

K.3.7 Breeding

K.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.

K.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 × 2,4 m are recommended.

K.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.

K.3.7.4 Detailed records should be kept of pedigrees as well as of fertility and rearing success.

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-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.

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.

K.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.

K.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.

K.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.

K.3.9 Handling

K.3.9.1 General

K.3.9.1.1 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.

K.3.9.1.2 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 sampling.

K.3.9.1.3 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.

K.3.9.2 Transportation

K.3.9.2.1 Transportation for swine is stressful and gentle handling is required.

K.3.9.2.2 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.

K.3.9.2.3 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.

K.3.9.2.4 If being transported over long periods, breaks should be instituted every 6 h where water is offered.

K.3.9.2.5 Swine should be transported free standing, with 0,5 m^2/100 kg space allowed.

K.3.9.2.6 Young, small swine can be transported in crates or dog kennels, with the floors covered in wood shavings. It may be advisable to transport larger swine under sedation, with the use of an alpha two agonist which can be reversed upon arrival at the research facility.

K.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.

Annex L
(informative)

Care and management of rabbits and guinea pigs

L.1 Introduction

L.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.

L.1.2 Rabbits

L.1.2.1 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. Females that were raised singularly may never allow another female into their territory. 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.

L.1.2.2 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.

L.1.2.3 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 well-being.

L.1.3 Guinea pigs

L.1.3.1 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.

L.1.3.2 All female groups rarely show aggression towards each other, if present it is of low intensity. 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. Pair housed males rarely exhibit agonistic behaviours, however if groups larger than two males are housed together this may not be true.

L.2 The environment

L.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.

L.2.2 Temperature

L.2.2.1 The optimal temperature range for housing rabbits is 16 °C to 22 °C, and 16 °C to 23 °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.

L.2.2.2 Reproductive performance can be significantly impaired if good temperature control is not maintained.

L.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.

L.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.

L.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.

L.2.2.6 It is essential that emergency equipment be available to maintain environmental temperatures, particularly in rooms that house small laboratory animals.

L.2.2.7 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.2.2.8 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 occasionally.

L.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.

L.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. During cleaning the humidity can temporarily spike to above 65 %.

L.2.4 Ventilation

L.2.4.1 To maintain suitable air quality, airflow rate requirements might differ depending on the type of housing, with tiered racks of cages likely to require higher rates than single-tiered open mesh cages or floor pens.

L.2.4.2 As rabbits shed considerable amounts of hair, the extract ducts should be cleaned regularly to ensure continued efficiency of ventilation.

L.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.

L.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.

L.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.

L.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.

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. 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.

L.2.5.2 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.

L.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).

L.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 (unless part of 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 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.

L.2.6.2 Sudden, irregular noises create more disturbances in rabbits and guinea pigs than continuous or predictable sounds.

L.2.6.3 Noise from fluorescent tubes such as infrasound and ultrasound should be avoided by fitting a type of light that is suitable for animal rooms. Ultrasound (above 20 kHz) is disturbing to guinea pigs, range for rabbits is not established.

L.2.7 Vibration

L.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.

L.2.7.2 Vibration stability will be of greater concern if the research animal facility is located on the upper levels of a building rather than at ground level because of structural considerations.

L.2.8 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). When on perforated floors, animals are to be provided with a portion of the cage that is solid flooring. This area should be at least 1/3 of the cage but should be able to accommodate all the animals simultaneously.

L.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.

L.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. Rabbits prefer straw, if possible this should be used.

L.3.2.4 Refuges, areas where the animals can move away from each other and hide after startling noises, are important for both guinea pigs and rabbits. There should be sufficient refuges for all the animals simultaneously to be hidden without agonist interactions.

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 rabbits and guinea pigs is strongly discouraged since this can be a source of intense stressful conflict. Animals are to receive an acclimatisation period of at minimum 7 days, longer if deemed necessary for health reasons or should scientific evidence indicate 7 days is insufficient. Positive interactions with humans should take place throughout the animal's life and the principles of positive reinforcement should be applied.

L.3.2 Disease prevention and health checks

Animals are to be monitored at least twice daily. Routine health checks for dental problems, parasites and infectious agents should be performed. The exact nature will depend on the study purpose, however the FELASA *Recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units* will provide a good reference. This protocol is to be discussed with the veterinarian. There are to be quarantine and isolation procedures of newly arrived or sick animals, to prevent infection of staff and or established animals. A full time or consultant veterinarian should be available to oversee the management of animals and health in the facility.

L.3.3 Food and water

L.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, either via dietary intake or water intake.

L.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.

L.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 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. Guinea pigs will play with water, given the chance and mess excessively. Ensure sufficient water is supplied to accommodate for this loss.

L.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 at different sites in the enclosure to avoid undue competition.

L.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.

L.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 diet that provides all required nutrients.

L.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. Nutrient loss by sterilization should be taken into account when determine feed levels and intake.

L.3.4 Cleaning

L.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. Toxicities of the agents with respect to the species housed should be considered and only agents known to not cause toxic changes within the animals can be used.

L.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.

L.3.5 Environmental enrichment

L.3.5.1 The welfare of rabbits housed in cages is enhanced by environmental enrichment, (for example, through the provision of hay, hay blocks or chew sticks).

L.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. Shelves or the use of vertical space provides opportunity for rabbits to jump. The use of straw for bedding and hay in the diet provides additional enrichment. Post-weaned animals should be maintained for as long as is possible, in compatible groups.

L.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.

L.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.

L.3.5.5 The facility is to have an enrichment programme which provides for the implementation of new ideas or current best practice to be considered. Provision of a rotation system for items which the animals tire of should be included, for example, balls for kicking (rabbits)

L.3.6 **Animal housing** (see tables 19 and 20)

L.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.

L.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 housing, where a wider behavioural and locomotor repertoire can be expressed. Study and care-planning should be aimed at allowing the group housing of social animal species.

L.3.6.3 Guinea pigs and rabbits need the social environment to guarantee their behavioural health and to safeguard their physiological well-being.

L.3.6.4 Solid floored cages are preferred. Mesh floors are only to be used under approval of the AEC and with provision in the cage of a solid area. 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. Animals on mesh floors are at a higher risk of developing pressure neuropathies. Heavy animals are prone to developing pressure sores and pododermatitis.

L.3.6.5 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. Perforated cage bottoms may cause sore hocks and abcessation of the hind feet.

L.3.6.6 A suitable substrate should be provided. Bare solid floors are not recommended 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.

L.3.7 Breeding

L.3.7.1 Nesting boxes should be provided for breeding does. Some substrate, for example, hay, straw or shredded paper, should be provided as bedding material. The box should be available for several days to a week before littering to permit the doe to exhibit normal nesting behaviour.

L.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.

L.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 of same sex should be housed in groups post-weaning. This facilitates subsequent group-housing programmes.

L.3.7.4 Does should be assessed for continued suitability for breeding before mating. The doe is to be placed in the buck's enclosure or a specific mating cage for mating. Does are territorial and may attack the buck if placed in its enclosure. Provide sufficient refuge that both animals are able to seek refuge at the same time.

L.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 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.

L.3.7.6 Disturbance of the animals should be minimized during late pregnancy and early lactation to reduce the risk of mismothering or cannibalism.

L.3.7.7 Detailed records should be kept of pedigrees as well as of fertility and rearing success.

L.3.7.8 The programme is to be in place outlining how the supply of animals will be matched to demand to minimize any surplus animals. This programme should highlight the handling, use and disposal of surplus animals. The AEC approval of this programme is required, and if requested animal numbers should be reported back to the AEC.

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 abcessation is minimal.

L.3.8.2 Permanent marking

L.3.8.2.1 Ear clipping provides an acceptable manner to number and thus uniquely identify guinea pigs, however guinea pigs may bite one another's ears and distort the clips.

L.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 sedation in as sterile conditions as possible, when the animals are fractious. Docile animals can have the chip implanted without sedation, working as sterily as possible.

L.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.

L.3.8.4 Temporary marking

A felt tip marker may be used for marking an ear, portion of the fur or tail base. This is easily applied but only lasts for 1 days to 2 days, 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.

L.3.9 Handling

L.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 technologist 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. Rabbits are never handled by their ears. When held in restraining devices for lengthy periods (hour or more), approval from the AEC is required and special monitoring of the animals required.

L.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.

L.3.9.3 To a considerable extent, proper handling depends on the handler rather than on the animal subject.

L.3.9.4 Personnel should wear appropriate personal protective clothing to minimize the exposure of staff to allergens and prevent transmission of disease between humans and animals.

L.3.10 Procedures

L.3.10.1 General

Procedures should be performed in a separate area outside of the animal room to prevent undue stress to the other animals in the room. Sedation, can be performed within a cage to minimize the impact on animal. The cage mates should be taken into consideration. Minor, non-invasive procedures such as weighing of the animals can be performed in the animal room.

L.3.10.2 Administration of substances / removal of bodily fluids

L.3.10.2.1 Personnel performing the administration should be trained in the procedure and until competent, under the direct supervision of a veterinarian with experience with the species.

L.3.10.2.2 The volumes to be injected for each site should not exceed the recommendations of the AEC. This is especially important for Intramuscular injection in guinea pigs and intra-dermal administration. The needle sizes should also not exceed the AEC recommendations. The volume for the removal of bodily fluids is to be stipulated in the AEC applications, but should be no more than 1 % of the animals circulating blood volume should be removed in a 14 day period.

L.3.10.2.3 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 specimens. Local anaesthetics creams that contain, for example, lidocaine and prilocaine at 2,5 % each, might serve the same purpose. The use of sedation is at the discretion of the veterinarian and as approved by the AEC.

L.3.10.3 Metabolic cages

L.10.3.3.1 The use of metabolic cages is subject to sound justification approved by an ethics committee.

L.10.3.3.2 Animals will require adaption to these cages, and any animal which is unable to cope should be withdrawn from use in the study.

L.10.3.3.3 The time kept within metabolic cages should be the kept to the absolute minimum.

L.10.3.3.4 Cage design should be of dimensions that the caging system has the least impact on the animal's welfare.

L.3.10.4 Telemetry

L.3.10.4.1 Habituation to the device, collar or jacket is imperative before use of the device.

L.3.10.4.2 Fitted or implanted devices should be of a size and weight that will have the least negative impact of the animal's health and welfare. Implantation should be performed with correct aseptic techniques and as needed, anaesthesia and sedation.

L.3.10.5 Anaesthesia, analgesia and perioperative care

L.3.10.5.1 When procedures are expected to induce pain or distress, appropriate anaesthesia and analgesia should be administered.

L.3.10.5.2 Multimodal approaches to analgesia are preferable. Pain and discomfort monitoring, including the response to analgesia, should be in place beforehand (as part of the study proposal). And staff adequately trained to perform these duties.

L.3.10.5.3 Pre-anaesthetic evaluation of the animal(s) health status and condition should be carried out.

L.3.10.5.4 Anaesthetic and analgesia protocols should be appropriate to the species, procedure and use.

L.3.10.5.5 Retrospective analysis of perioperative care should be performed in order that any improvements and recommendations can be incorporated into future studies.

L.3.10.6 Transport

L.3.10.6.1 Transport of animals should take into consideration the temperature the animals are acclimatised too and the temperature during transport.

L.3.10.6.2 Animals should be transported via the shortest route (time) and at the cooler time of day. In a vehicle without direct sunlight on the animals (especially important for albino species). For additional requirements on the transportation of animals, see 10.2.

L.3.10.7 Recognizing and monitoring adverse effects

L.3.10.7.1 In line with refinement principle, pain should be avoided, alleviated and prevented. A list of likely or expected adverse effects should be included in the study protocol, with methods of monitoring and mitigating treatments described for each.

L.3.10.7.2 Staff is to be trained in these methods before a study starts to ensure adverse events will be recognized, mitigating measures implemented and the event reported to veterinary personnel. Records of such events should be kept and any interventions documented.

L.3.10.7.3 Retrospective analysis of monitoring methods, treatments, clinical procedures and perioperative care should be performed and improvements and recommendations incorporated into future studies.

L.3.10.8 Euthanasia

L.3.10.8.1 Euthanasia methods should conform with current ethical standards.

L.3.10.8.2 Methods considered to be acceptable include:

a) Injection of an overdose of a barbiturate and barbituric acid derivatives, intravenous administration is preferred to intraperitoneal. Fractious animals may require sedation to obtain IV access.

b) Inhalation of anaesthetic gases such as halothane, sevoflurane, isoflurane, nitrous oxide are acceptable (nitrous oxide cannot be used alone). These methods are conditional to the correct flow rate, compatible groups in the chamber. Inhalation methods in rabbits should only follow after sedation of the animal due to their breath-holding tendencies.

c) Inhalation of carbon dioxide only with a flow rate of 10 % to 30 % displacement of the chamber volume per minute. Death should be verified. The chamber should be cleaned between cages should the home cage not be used. In rabbits CO_2 euthanasia should only follow after sedation of the animal due to the distress it induces.

d) Penetrating captive bolt (rabbit-sizes) is acceptable for rabbits if the bolt is maintained in a clean working order, positioned correctly and operated correctly.

e) Physical methods such as cervical dislocation need a high level of technical competency. These are acceptable if this competency can be achieved, however, only under sound justification and approval of the AEC.

L.3.9 Records

L.3.9.1 Each rabbit and guinea pig should be individually identifiable and individual records should be kept.

L.3.9.2 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.

Annex M
(informative)

Care and management of rodents (mice, rats and hamsters)

M.1 Introduction

M.1.1 General

The 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.

M.1.2 Mouse

M.1.2.1 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.

M.1.2.2 Mice are capable of assuming a wide range of social organizations and intense territoriality might be seen in reproductively active males.

M.1.2.3 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.

M.1.2.4 Mice are very social animals and group housing of females should be done where possible.

NOTE Cognisance can be placed in on their social behaviour and special note that highly specialized strains have different behaviours due to selective breeding needs to be placed in.

M.1.3 Rat

M.1.3.1 As the rat is a much more social animal than the mouse, disruption to social groups should be minimized. Young animals are very exploratory and interact to an enormous degree.

M.1.3.2 Rats are excellent climbers, therefore, avoid open spaces and use urine spotting as a territorial marker. Their sense of smell and hearing are highly developed, and they are particularly sensitive to ultrasound.

M.1.3.3 Since rats are social animals, cognisance should be given to allow for adequate adult stimulation; ability to see and smell other rats in the same room. Daylight vision is poor, but dim-light vision is effective in some pigmented strains. Activity is higher during hours of darkness.

M.1.4 Hamster

M.1.4.1 The hamster species is very different from the mouse and the rat. The female is larger and more aggressive than the male.

M.1.4.2 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.

M.1.4.3 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.

M.1.4.4 Hamsters use their mouth pouches to store food so care needs to be taken to ensure animals do not develop infection from sharp edged food items.

M.1.4.5 Careful control of environmental features and prevention of disruption during routine husbandry practices are of particular importance in this species.

M.2 The environment

M.2.1 General

M.2.1.1 The laboratory rodents are species that choose to manipulate their own micro-environments 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.

M.2.1.2 For properly maintained animal rooms, there should be back-up power and adequate consideration for additional back-up supply of temperature and humidity.

M.2.1.3 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).

M.2.1.4 Micro-environment 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.

M.2.2 Temperature

M.2.2.1 The optimal temperature range for mice, rats and hamsters is 20 °C to 24 °C.

M.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.

M.2.2.3 Provision of bedding or nesting material allows the animal an opportunity to manipulate its own immediate environment, provides a warm nest for its young and may also prevent cannibalism of the young.

M.2.2.4 It is essential that emergency equipment be available to maintain environmental temperatures, particularly in rooms housing small laboratory animals.

M.2.2.5 In special cases, for example, when housing very young or hairless animals, higher room temperatures than those indicated in M.2.2.1 might be required.

M.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 calibrated 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.2.2.7 Data loggers may also be used, these are read out at certain intervals. Rooms should also be connected to a BMS (Building Management System) that will indicate an alarm if any of the parameters is outside their limits.

M.2.2.8 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

M.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. Humidity should be monitored continuously with data loggers.

M.2.3.2 Since a low relative humidity might contribute to the development of ringtail in rats, levels of less than 40 % should be avoided.

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 15 exchanges to 20 exchanges per hour, for rooms with open cages, 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.

M.2.4.4 When ventilated cages are in use (individually vented cages) the air exchanges in the room can be lowered to 10 air exchanges. Air exchanges in the room should be validated at a minimum once a year, unless major environmental changes have occurred in or around the animal rooms in question.

M.2.4.5 When applicable, 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.

M.2.4.6 Differential pressures can be used to inhibit the passage of pathogenic material between rooms. Higher pressures can be used to protect clean areas, as opposed to dirty or biohazardous ones, in order to minimize contamination. Under Biosafety Levels (BSL) when working with infectious diseases, the unit may be protected by negative pressure. 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 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.

NOTE Florescent tubes need to be checked every six months, as they become less bright with use.

M.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.

M.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.

M.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 or houses, tunnels).

M.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, these should be monitored daily. 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 breeding rodents than continuous or predictable sounds.

M.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.

M.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 – especially rodents.

M.2.7 Vibration

M.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.

M.2.7.2 Vibration stability will be of greater concern if the research animal facility is located on the upper levels of a building rather than at ground level because of structural considerations. Excessive vibration may influence breeding in rodents.

M.3 Animal care and health

M.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.

M.3.2 Bedding and nesting material

M.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 or grid above the bedding or perforated bottom cages).

M.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. This might also promote greater utilization of the available space, provide enrichment and mitigate abnormal behaviour such as cannibalism of the pups.

M.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.

M.3.2.4 Preferably animals should not be housed in wire cages as this increases their chance of foot injuries like pododermatitis and other injuries. When wire cages are in use the animals have to be provided with a rest area that has sold flooring for example nesting boxes or plastic trays.

M.3.3 Food and water

M.3.3.1 Since water differs in its quality (contamination, chlorination, oxidation, etc.), poor quality water can influence animal studies adversely. Potable water should be supplied to animals in sufficient quantity and be presented in a manner that an animal can use. Municipal tap water might be sufficient for conventional housing facilities, but for SPF or barrier units, water should be sterilized.

NOTE It is always advisable to test municipal water for its quality.

M.3.3.2 Water sterilization is easily achieved by autoclaving the filled water bottles, acidifying water to a pH value of 2,5 or properly filtering the water. This procedure should be carefully controlled and taken into account as a study variable. Mice might adapt to the change from municipal tap to acidified water through time.

M.3.3.3 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.

M.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.

M.3.3.5 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 feed should be a balanced diet that provides all required nutrients, and be bought from a reputable animal feed supplier.

M.3.3.6 For rodents feed that need to be sterilized this can be facilitated by irradiation or autoclaving.

NOTE Pre-treatment of food may result in the loss of nutrients and adequate care should be taken to prevent this from happening.

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. Care should be applied in the choice of detergents, as the smell of the produce may adversely affect the health of the animal.

M.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.

M.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.

M.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.

M.3.5 Environmental enrichment

M.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. Numerous other enrichment aids can be used such as, but not limited to, cardboard toilet roll inners, egg cardboard cartons, propylene containers, mouse houses and tissues.

NOTE It is advisable that bedding and enrichment aids are sterilized before use.

M.3.5.2 Since these rodent species are generally social animals, disruption of established groups should be minimized as this can be very stressful.

M.3.5.3 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. Animals need to be monitored for uneven ware on their teeth, so that appropriate veterinary treatment may be provided.

M.3.6 Animal housing (see tables 21 and 22)

M.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.

M.3.6.2 Young animals should be maintained in compatible groups.

M.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. When animals are singly housed, they need to be provided with adequate enrichment aids. Typically, single housed animals should receive two enrichment aids whereas group housing can allow for one.

M.3.7 Breeding

M.3.7.1 Rodents should be bred on solid floors and be provided with suitable bedding material, such as shredded paper or wood chippings or shavings, from which a nest can be constructed. This is important in the thermoregulation of the micro-environment, and keeps the young together for efficient lactation. Nestlets and tissues may also be provided to encourage rodents to build nests as well as for thermoregulation.

M.3.7.2 Disturbance to the animals should be minimized during late pregnancy and early lactation to reduce the risk of mismothering or cannibalism.

M.3.7.3 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 Toe, ear and tail clipping

M.3.8.2.1.1 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.

M.3.8.2.1.2 When toe clipping or tail docking are felt to be the only methods that can meet the requirements of a particular study, their use should 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.

M.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.

M.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.

M.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 day to 2 days, 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.

M.3.9 Handling

M.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.

M.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 before restraining. To a considerable extent, proper handling depends on the handler rather than on the animal subject.

M.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.

M.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.

M.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. Gloves should be worn when working with rodents, especially if the animals are SPF.

M.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.

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 rodent units, or where large numbers of animals are maintained in breeding colonies, or where there is a high animal turnover.

Annex N
(informative)

Care and management of sheep and goats

N.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.

N.2 The environment

N.2.1 General (outdoors)

N.2.1.1 Sheep and goats are capable of adapting to adverse climate conditions. For reasons of providing standardized research environments, these animals are occasionally stabled in environmentally- controlled facilities.

N.2.1.2 If sheep and goats are housed outdoors, they may 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.

N.2.2 Temperature (indoors)

N.2.2.1 Sheep and goats housed indoors should generally be maintained at ambient temperatures.

N.2.2.2 In special cases, for example, when housing very young or recovering animals, higher or lower temperatures than ambient might be required. Gradual acclimatization may be necessary before moving them outdoors after they have either been shorn or adapted to indoor conditions.

N.2.2.3 Temperature should be monitored daily, where necessary, by continuous recording. The use of a maximum and minimum thermometer that is examined and reset daily may be adequate for most circumstances. The temperature of the microenvironment, at the animal level, should also be monitored.

N.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.

N.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 30 % to 70 % is preferable provided that the temperature range is appropriate.

N.2.4 Ventilation

N.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. Particular care should be taken to limit dust and ammonia concentrations, at animal level, within the holding rooms.

N.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.

N.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.

N.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.

NOTE Properly designed passive ventilation measures are usually adequate to meet the needs of small ruminants.

N.2.5 Lighting

N.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.

N.2.5.2 Where natural lighting is not used, light and dark periods should be at least 6 h each per day.

N.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.

N.2.6 Noise

N.2.6.1 Sudden, loud irregular noises create more disturbances in sheep and goats than continuous softer or predictable sounds.

N.2.6.2 Noise cannot be eliminated from an animal unit but care should be taken to minimize the generation of sudden extraneous high volume, audible and ultrasound noise in the vicinity of animals.

N.2.7 Vibration

N.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.

N.2.7.2 Vibration stability will be of greater concern if the research animal facility is located on the upper levels of a building rather than at ground level because of structural considerations.

N.3 Animal care and health

N.3.1 General

N.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 social animals which depend on social contact and will show severe stress reactions if separated from their flock or herd. If individual housing is required, the animals should at least have visible contact with conspecifics.

N.3.1.2 Sheep and goats display a leader-following behaviour. It is thus recommended to encourage this behaviour, for example, by moving them through gangways or scales instead of coercing the animals by means of force or fear, which usually results in chaos.

N.3.1.3 Sheep and goats also respond well to positive food reinforcement such as the provision of concentrates. Low stress handling can be achieved by competent, calm and confident personnel within an environment that is designed to assist such efforts.

N.3.1.4 Supervision and inspection of animals should be appropriate for the conditions under which the animals are kept.

N.3.2 Bedding material

N.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.

N.3.2.2 Bedding may be non-nutritive, but should be non-toxic, absorbent and comfortable. Resinous wood shavings, especially from coniferous wood, are not suitable for use as experimental animal bedding.

N.3.2.3 Slatted floors or cages with grates or perforated bottoms require special caution, and should be properly constructed and maintained. 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.

N.3.3 Feed and water

N.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 be spaced to avoid head trapping particularly with horn animals.

N.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.

N.3.3.3 Sheep and goats may soil their water with faeces, urine or food (or both). Water and feed troughs should be cleaned on a frequent basis, preferably twice a week when soiled.

N.3.3.4 Feeding and watering space should be constructed to minimize chances of contamination by urine or faeces. The feeding and watering points should allow for the number of animals being kept.

N.3.3.5 An individual animal's nutrient requirements are affected by many factors. The quantity and quality (macro and micro nutrients) of the feed provided should be appropriate to the requirements of the animal in the experiment. Environmental factors can also affect food intake and nutrient needs.

N.3.3.6 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.

N.3.3.7 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.

N.3.3.8 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.

N.3.3.9 Body weight changes may be useful to monitor nutritional adequacy.

N.3.4 Cleaning

N.3.4.1 Routine cleaning and maintenance, and a high standard of hygiene are essential for good husbandry, especially for intensively housed animals. Suitable and institutionally approved cleaning agents and procedures should be applied.

N.3.4.2 The facilities should be designed to support manure removal, cleaning and disinfection.

N.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.

N.3.4.4 Fly and other pest populations should be regularly monitored and appropriate control measures be applied when indicated.

N.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 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.

N.3.5.1 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.

N.3.5.2 The successful application of the use of positive reinforcement for sheep and goats has been described in Hutson, 1985 and Hargraves and Hutson, 1990. Where possible the natural behavioural requirements of the animal should be met, especially in intensively housed conditions.

N.3.6 Animal housing (see table 23)

N.3.6.1 Sheep and goats housing facilities should provide suitable access and restraining devices to allow animals to be inspected, caught or moved as necessary.

N.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.

N.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.

N.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.

N.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. When in other pens where sheep or goats are kept for short periods, enough space should be allowed for all animals to stand comfortably. For the transport of animals see 10.2.

N.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.

N.3.7 Breeding

N.3.7.1 Ewes and does should be allowed to give birth with minimum interference. Animal attendants should be familiar with normal birth process and should be able to recognize if intervention is necessary. Assistance at birthing should, if necessary, be provided by capable trained attendants. Where veterinary assistance is required, a veterinarian should be called without delay.

N.3.7.2 New born lambs and kids require adequate nutrition and a high level of hygiene. Mothers and their offspring should be disturbed as little as possible.

N.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.

N.3.8 Animal identification

N.3.8.1 General

N.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.

N.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.

N.3.8.2 Permanent marking

N.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.

N.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.

N.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.

N.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.

N.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.

N.3.8.4 Temporary marking

Sheep and goats can be marked with marking sticks or stock marker spray-paint, 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.

N.3.9 Handling

N.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, by the use of a crook or by the hind leg above the hock. 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.

N.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 acceptable. Goats should be restrained with one hand under the jaw and one hand over the head. The sitting technique used with sheep (see N.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.

N.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.

N.3.10 Animal healthcare

N.3.10.1 Animals should be inspected for health and welfare, at appropriate regular intervals.

N.3.10.2 Timely appropriate action should be taken when problems are identified.

N.3.10.3 An appropriate preventative health programme should be in place.

N.3.10.4 Where treatment is not indicated the animal should be euthanized without delay using appropriate equipment.

N.3.10.5 Regular record keeping 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.

Annex O
(informative)

Care and management of terrestrial reptiles

O.1 General

O.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 water loss and from the absorption of noxious substances from their environment.

O.1.2 Virtually all major groups of reptiles contain some Threatened and Endangered species. National and international conservation regulations should 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.

O.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.

O.1.4 It is advisable to maintain the different species separately and keep the numbers held in primary enclosures to a minimum.

O.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.

O.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.

O.3 Behavioural interactions

O.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.

O.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.

O.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.

O.5 Animal housing (see 10.7.13)

O.5.1 Housing for reptiles

O.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.

O.5.1.2 Ideally, a species-specific designated holding room is required. However, the number of reptiles held in an research 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.

O.5.1.3 Small snakes do not do well in large cages. Large snakes do not do well in small cages.

O.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.

O.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.

O.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.

O.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.

O.5.1.8 Aromatics wood shavings (for example, pine) can be toxic to snakes and should not be used.

O.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 should 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 day to 2 days to avoid faecal contamination and bacterial build-up.

O.5.2 Aquatic holding systems for fresh water turtles

O.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.

O.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.

O.5.3 Housing for venomous snakes

In addition to general housing requirements (see O.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 should have suitable handling experience.

f) **Antisera** – The institution, research animal facility and all medical staff should be informed of the type(s) of snakes held or being introduced.

O.6 Skin and scales

O.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.

O.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.

O.6.3 Lizards, turtles and crocodiles shed in many pieces.

O.7 Diet, UV radiation and mineral imbalances

O.7.1 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).

O.7.2 All of these situations can give rise to rickets, a soft shell, and a predisposition to disease.

O.8 Size range and lifespan

O.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.

O.8.2 Smaller reptiles have a shorter lifespan. The 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 100 years.

O.9 Reproduction and egg-laying

O.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.

O.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.

O.9.3 Egg-laying females will bury their eggs in soil or sand, rock crevices, or under bark.

O.9.4 Eggs are oval or round, and the shell covering can be hard (calcium salts) or relatively soft and leathery.

O.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.

O.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) should be complied with.

O.11 Temperature and light

O.11.1 General

O.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) should be avoided.

O.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.

O.11.1.3 Optimal temperatures for reptiles are 25 °C to 30 °C, and for lizards up to 35 °C.

O.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.

O.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.

O.11.1.6 A regular light cycle should always be maintained, or be as determined by approved study requirements.

O.11.2 Ultraviolet radiation

O.11.2.1 Where exposure to natural sunlight is not possible, UV radiation should be supplied.

O.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 should 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 should benefit from them.

d) Broad-spectrum tubes are of limited usefulness. Middle and long wavelength lights are optimal.

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.

O.12 Humidity and ventilation

O.12.1 Reptiles are better able to prevent water loss 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.

O.12.2 Control of air exchange in the terrarium as well as the holding room should be provided. Ventilation ports should be screened to allow airflow and prevent escape.

O.12.3 Excessive humidity can lead to a predisposition to diseases of the respiratory system, skin, scales, and shells of tortoises.

O.13 Refugia

O.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.

O.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.

O.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.

O.13.4 A raised flat surface, with a heat source for basking on, should be provided for all reptiles.

O.14 Servicing and viewing

O.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.

O.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.

O.15 Sanitation

O.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.

O.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.

O.15.3 Cages should be impervious to water and cleaning agents should 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.

O.16 Water, food and feeding

O.16.1 General

O.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 O.5.1.9).

O.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.

O.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.

O.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).

O.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.

O.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.

O.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.

O.16.1.8 Many reptile illnesses are caused by improper diet or food preparation.

O.16.2 Carnivorous turtles

O.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.

O.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 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.

O.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.

O.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.

O.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.

O.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.

O.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.

O.19 Diseases and parasites

O.19.1 General

O.19.1.1 Many snakes and reptiles are easy to keep. However, animals caught in the wild can carry viral, bacterial and parasitic diseases.

O.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 weight loss.

O.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.

O.19.1.4 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.

O.19.1.5 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.

O.19.1.6 The following infections can occur:

a) ***Salmonella*** – *Salmonella* is carried by many reptilians and is a common and potentially serious zoönotic 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.

O.19.1.7 Newly introduced animals from the wild or other sources should be tested and quarantined for a minimum of 14 days.

O.19.2 Mite and tick infestations

O.19.2.1 Mites and ticks 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.

O.19.2.2 Mites can be treated with a topical insecticidal dust, or application or injection of ivomectin. Substrates should 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.

O.19.2.3 Ticks are often found attached to soft skin areas between scales and may be removed manually.

O.19.3 **Mouth rot** (necrotic stomatitis)

O.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.

O.19.3.2 Changing the substrate is recommended.

O.20 Handling and restraint

O.20.1 Appropriate handling equipment and protective clothing, gloves, goggles, plastics tubes, hooks and tongs should be available onsite.

O.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.

O.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.

O.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 ± 1 m above the ground and transferred.

O.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.

O.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.

O.21 Euthanasia

O.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.

O.21.2 Acceptable methods are exposure to halothane, methoxyflurane and injection of sodium pentobarbitone. Death should always be confirmed.

O.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.

Annex P
(informative)

Pain management and humane endpoints

P.1 General

P.1.1 When conducting experiments that could involve a great deal of animal distress, one should consider the option of implementing humane endpoints.

P.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 weight loss. 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.

P.1.3 When preparing a project application for all but the most minor manipulations, the researcher or the 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.

P.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).

P.3 Types of criteria for humane endpoints

Humane endpoints can be based on different types of parameters (see table P.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.

Table P.1 — Some indicators used to justify humane endpoints

1	2	3
Clinical behaviour	**Pathophysiological indicators**	**Biomedical or hormonal indicators**
Activity	Respiration rate	Acute phase proteins
Aggression	Complete blood count	Cathecholamines
Posture	Weightloss	Corticosteroids
Response to handling	Heart rate	Glucagon
Vocalization	Dehydration	Insulin
	Anuria	Prolactin

P.4 The use of humane endpoints in research projects

P.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 post-mortem procedure.

P.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.

P.5 Humane endpoints and actions to be taken

P.5.1 Humane endpoints (see 3.16) 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).

P.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.

P.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 seven days or loses more than 20 % of its initial weight at any time, or

c) an animal shows tiptoe or slow ponderous gait.

P.5.4 If more than one clinical sign occurs, then the veterinarian and principal researcher should be informed.

P.6 Responsibility

P.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 post-mortem 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).

P.6.2 In a case of uncertainty, expert advice should be obtained, normally from a laboratory animal scientist, veterinarian, or a pathologist.

P.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.

P.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.

P.8 Recognition

P.8.1 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 well-being is being affected, both positively and negatively. Recognition can be considered as a fourth Rs, following on after the three As (Avoidance, Assessment and Alleviation) of animal suffering in research.

P.8.2 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 Claassen, 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.

P.9 Development and validation of humane endpoints

P.9.1 Planning considerations

P.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.

P.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.

P.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 P.2(a) and P.2(b)) should be kept in mind.

P.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.

P.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.

P.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 P.9.3(a); and

c) assurance of the scientific validation (i.e. it satisfies to a large degree the criteria in P.9.2).

P.10 The welfare assessment and the use of the score sheet system

P.10.1 General Principles for an effective welfare assessment

P.10.1.1 A team approach – A team approach is the most effective way to ensure consistency and effectiveness. The team should include people with a variety of relevant roles and expertise who are prepared to work together constructively.

P.10.1.2 Appropriate welfare indicators – An animal's welfare state cannot be directly measured, but it can be inferred by monitoring appropriate behavioural and physiological parameters that can be used as welfare indicators. It is critical to define and monitor the right types and number of indicators – too many and the system will take too long to implement, too few and it may be inaccurate and misleading.

P.10.1.3 A sound understanding of good welfare and the ″normal″ animal – Effective welfare assessors should be able to recognize a ″normal″ animal, with good welfare, in order to detect early signs of adverse effects. However, the definitions of both ″good welfare″ and ″normal″ need to be very carefully considered.

P.10.1.4 Full recognition of all potential adverse effects from all sources – There are many potential causes of adverse effects during the animals' lifetime, i.e. not just the scientific procedures but other factors such as husbandry, handling and transport. An effective welfare assessment scheme will consider all sources of potential harms and all the adverse effects associated with them.

P.10.1.5 Consistency for all species – Ideally, welfare assessment protocols should pay the same level of attention to all species, regardless of the numbers of animals used.

P.10.1.6 Consistency between observers – Minimizing variation between assessors' observations is essential. Differences in observational skills and subjective interpretations can be reduced by effective training and teamwork, and also by ensuring that observations are adequately described and recorded in a meaningful way.

P.10.1.7 Appropriate recording systems – There are a number of different systems for recording welfare assessment data, each of which has particular advantages and disadvantages that make it suitable for use in different situations. Data should be captured using a consistent language and format, with the most appropriate recording system for each establishment, species, project and group of personnel.

P.10.2 Practical welfare assessment

P.10.2.1 Making observations

P.10.2.1.1 Having the same person, or a very small number of people, observing the animals wherever possible can facilitate consistency and enable assessors to follow the progression of an animal's condition more accurately.

P.10.2.1.2 Animals may also be able to tell the difference between different people so they may benefit from contact with familiar staff as opposed to strangers.

P.10.2.1.3 Consistency of staff also enhances job satisfaction for carers, many of whom prefer to be responsible for the same animals throughout a study.

P.10.2.2 Observation from a distance

P.10.2.2.1 Assessing the general appearance, posture and behaviour without provoking any responses provides useful information. So animals should first be observed from a distance without moving, approaching or entering the enclosure or opening the cage. This enables the observer to see whether there are unprovoked behaviours that could indicate welfare problems, such as social animals isolated from conspecifics, or nocturnal animals immobile but out of the nest. Alternatively, individuals may be playing, foraging or grooming, indicating that welfare is probably good.

P.10.2.2.2 The enclosure should also be observed to see whether activities such as nest building, foraging or gnawing are reduced, or whether there is evidence of health problems including bleeding, vomiting or abnormal faeces. Clinical or behavioural indicators such as piloerection, postural changes, reduced mobility or favouring a surgical site in any species should be noted. These signs may be either specific to the project or unexpected, in which case they should be entered into the additional observation box on the score sheet.

P.10.2.3 Opening the cage or entering the enclosure

The next stage is to examine the enclosure in more detail by removing the cage lid or entering the enclosure or closely approaching it as appropriate. Enrichment items or nesting material should be removed or moved aside if necessary, and the animals' reactions to this should be watched for a suitable period of time, as previously determined. Most species would normally respond with increased activity followed by a settling down period. Any specific or unexpected signs should be recorded.

P.10.2.4 Handling the animals

After completing the above initial checks, the animals should be individually handled and clinically examined in accordance with the pre-determined set parameters in order to measure and score relevant criteria such as body weight, body condition and temperature. This is also the time to assess those specific criteria that require handling, such as skin tenting, sensitive areas, tumour measurement or parameters that require blood sampling. Handling is stressful for many animals, which will then affect both animal welfare and the scientific data. Observing animals following handling can facilitate observing some relevant behaviours, such as the post-surgical behaviours in order to reduce the time needed for the assessment.

NOTE It is not always appropriate to handle animals. Handling may cause discomfort or pain following certain procedures, or removing animals from their housing would cause excessive distress under some circumstances.

P.10.3 Score sheets

P.10.3.1 A score sheet can be an essential instrument in determining humane endpoints. It is a form on which the clinical status of each individual experimental animal is recorded at regular, pre-determined intervals. Over time, this score sheet will provide an overview of the health status of an animal.

P.10.3.2 For every scientific procedure, a separate score sheet should be designed and the specific clinical findings of the experiment should be recorded on this sheet. For new procedures or investigations, these findings may be unknown requiring that the assessment of the animal's condition covers a wide range of clinical aspects. For familiar procedures or investigations, the assessment can be limited to the relevant clinical parameters and possible unusual findings.

P.10.3.3 Analysis of the score sheet will show the pattern of recovery or deterioration of each animal's condition over time and the effect of the procedure on the animal's health.

P.10.3.4 The use of score sheets should be considered at the project planning stage.

P.10.3.5 Score sheets should be developed that are specific and adapted for each individual research protocol.

P.10.3.6 Score sheets should be as simple as possible, but as detailed as needed, and tailored to the type of study.

P.10.3.7 Previously developed assessment sheets can be used if these are appropriate to the study, species and strain.

P.10.3.8 The use of standardized language and terminology is recommended.

P.10.3.9 The data recorded should be as objective as possible.

P.10.3.10 All types of observation record should include a facility to add additional observations, as well as predetermined indicators, so that unexpected observations can be recorded.

P.10.3.11 Effective training for all relevant staff is essential covering specifically severity and welfare assessment as well as monitoring techniques.

P.10.3.12 A communication plan should be established to encompass all relevant staff; this should include a mechanism to rapidly communicate unexpected outcomes to all appropriate individuals.

P.10.3.13 Monitoring should be proportionate to anticipated effects – procedures that may cause ″severe″ suffering will generally require more frequent and detailed monitoring.

NOTE There should be clear criteria for intervention, for example, if particular parameters are observed or if a predetermined level of suffering is approached. All relevant staff should know what these criteria are, know what to do and whom to contact should they occur.

P.10.4 Types of score sheet systems

P.10.4.1 Numerical score sheets – Clinical signs, physical indicators and behavioural parameters are assessed and given a score in accordance with their apparent severity. For example, unaffected would score 0, mild deviation from normal might score 1, moderate deviation from normal 2 and substantial deviation 3. Scores are then added up and the results are used to determine whether an action or an intervention is needed (such as analgesia), in accordance with a predetermined key attached to the sheet.

P.10.4.2 Binary score sheets – This score sheet system records either a yes (present) or a no (absent) depending on whether the behaviour or effect is seen or not, with no description of its intensity.

P.10.5 Score sheets and pilot studies

P.10.5.1 Pilot studies using a small number of animals can provide useful guidance on welfare indicators where these are difficult to predict, to be included in score sheets. For example, for the testing of novel compounds or for the newly developed experimental designs.

P.10.5.2 The results of pilot studies cannot only provide the indicators for the final project, but also help to guide refinements, including intervention points and humane endpoints.

P.10.5.3 The first animals in a pilot study should be monitored extremely carefully, using a broad range of indicators, so as to gain as much information as possible about potential adverse effects and their progression.

P.10.6 Advantages of score sheets

P.10.6.1 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.

P.10.6.2 Subjective assessments of suffering by the staff and the researchers are avoided; thereby promoting more fruitful dialogue, as evidence based on opinion becomes possible supported by the clinical proof.

P.10.6.3 Consistency of scoring is increased as the guidance is clear and the scoring options are limited.

P.10.6.4 The score sheet helps to determine the effectiveness of any therapy intended to relieve adverse effects.

P.10.6.5 The score sheet can be used to analyze retrospectively the adverse effects of any scientific procedure and its severity level.

P.10.6.6 The score sheet has been found to add to the scientific study as a more careful observation of animals is carried out.

P.10.6.7 The score sheet 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.

P.10.7 Study specific score sheets – General example templates for various scientific research studies or models

P.10.7.1 Annex 1: Score sheet – Arthritis

P.10.7.2 Annex 2: Score sheet – Post surgical

P.10.7.3 Annex 3: Score sheet – Toxicity study

P.10.7.4 Annex 4: Score sheet – Oncology study

P.10.7.5 Annex 5: Score sheet – Stroke study

P.10.8 Principles to note when using these examples of score sheets

P.10.8.1 Good practice standards and principles with respect to housing, husbandry and care are implemented in accordance with institutional and organizational SOPs, institutional and organizational training and education by the assessment of competence while applying current information regarding the principles of replacement, reduction and refinement.

P.10.8.2 The score sheets that are provided are examples and should be adjusted for each specific scientific research protocol and should be revised and adjusted as knowledge increases and observations are made regarding indicators for pain, suffering and distress.

P.10.8.3 As severity of welfare indicators such as behavioural or clinical signs increase, welfare monitoring will be increased accordingly so as to ensure timeous interventions and actions and implementation of pre-determined humane endpoints in order to reduce unnecessary distress.

NOTE The developers of the study specific score sheet need to continually confirm the actual severity scores of the welfare indicators. This should be done by defining "mild, moderate and severe" levels for each of the indicators used in the day to day assessments and then making a judgement about the severity of these indicators on a case by case basis.

ANNEX 1: ANIMAL WELFARE SCORE SHEET: ARTHRTIS STUDY PROTOCOL NUMBER:

SHORT TITLE:

Animal ID: Baseline body weight: Max body weight gained: Humane end Point body weight (%)

Date of last treatment/procedure: Monitoring Frequency:

CATEGORIES	INDICATORS	SCORE
DATE		
BODY WEIGHT		
APPEARANCE	Normal - no weight loss	0
	5 % - 10 % weight loss	1
	11 % - 15 % weight loss	2
	16 % - % 20 % weight loss	3
	20 % weight loss	HEP
	Lack of grooming	1
	Pinched skin/dehydration	1
BODY FUNCTIONS	Dyspnoea (Difficulty breathing)	2
	Tachypnoea (fast breathing)	1
BEHAVIOUR	Reluctance to move	1
	Lethargy/apathy	2
	Persistent immobility <24h	3
	Immobility >24h	HEP
	Vocalization on handling	1
	Vocalization, tense and nervous on handling	2
	Vocalization on moving/spontaneous	3
PROCEDURE SPECIFIC INDICATOR	Normal	0
ARTHRITIC PAW SCORE	Erythema and swelling of one ankle	1
Score as the sum of both paws 0-4	Erythema and swelling of ankle and proximal half of tarsal joints	2
	Erythema and swelling of ankle and all tarsal joints up to metatarsal joints	3
TOTAL SCORE		
Additional Observations		

Actions / Interventions to be taken	Total score
Increase frequency of monitoring; consider supplementary fluids/care	≥4
Review progress with veterinarian/animal welfare manager	5-15
Implement Humane-endpoint	HEP AND/OR

ANNEX 2: ANIMAL WELFARE SCORE SHEET: POST SURGICAL **PROTOCOL NUMBER:**
SHORT TITLE:

Animal ID: Baseline body weight:

Max body weight gained: Humane end point body weight (%)

Date of last treatment/procedure: Monitoring Frequency:

CATEGORIES	INDICATORS	SCORE
DATE		
BODY WEIGHT		
APPEARANCE:BODY WEIGHT	≤ 5% weight loss	0
	6-10% weight loss	1
	11-20% weight loss	2
	≥ 20% weight loss	HEP
COAT CONDITION	Normal	0
	Coat slightly unkempt	1
	Moderate piloerection	2
	Severe piloerection	3
BODY CONDITION	Well-conditioned	0
	Under conditioned	2
	Emaciated	3
POSTURE	No huddling or hunched posture	0
	Mild hunched body posture	1
	Hunched posture and twitching	2
	Huddling, hunched posture and twitching	HEP
BEHAVIOUR	Normal cage exploration, responsive and inquisitive	0
	Minimal cage exploration but responsive and shows investigative behavior	1
	No cage exploration, reluctant to move	2
	Non-responsive, severely depressed, immobile	3
CLINICAL SIGNS RESPIRATION	Normal rate and depth	0
	Rate and depth slightly increased	1
	Depth – increased respiratory effort visible	2
	Dyspnoeic with pale/cyanotic extremities	3
GAIT	Normal	0

CATEGORIES	INDICATORS	SCORE
	Slight inflammation and redness visible, sutures intact	1
	Severe inflammation and redness with wound interference	2
	Infection present – discharge and severe inflammation	3
WOUND PALPATION	No discomfort	0
	Discomfort and wriggling	1
	Discomfort with aggressive response	2
	Severe discomfort and vocalization	3
PAIN SCORE GRIMACE SCALE	Orbital Tightening	0-2
Not present =0	Nose Bulge	0-2
Moderately visible = 1	Cheek Bulge	0-2
Severe = 2	Ear Position	0-2
	Whisker Change	0-2
TOTAL SCORE		
Additional Observations		

Actions / Interventions to be taken	**Total score**
No intervention- re-check in 8-12 hours (twice daily).	0
Re-check in 1-2 hours	1-2
No improvement from 1-2 hours ago: start analgesia	≥ 3
Progress to next tier of analgesic regime & re-evaluate after 1	≥10 with no
Humane endpoint	3 is scored in 2 or more categories

ANNEX 3: ANIMAL WELFARE SCORE SHEET: TOXICITY STUDY

PROTOCOL NUMBER:
SHORT TITLE:

Animal ID: Baseline body weight: Max body weight gained:

Humane end point body weight (%)

Date of last treatment/procedure: Monitoring Frequency:

CATEGORIES	INDICATORS	SCORE
DATE		
BODY WEIGHT		
APPEARANCE: BODY WEIGHT	5 % - 10 % weight loss	1
	11 % - 15 % weight loss	2
	16 % - 20 % weight loss	3
	20 % weight loss	HEP
COAT CONDITION	Coat slightly unkempt	1
	Slight piloerection	2
	Marked piloerection	3
BODY CONDITION	Well-conditioned	0
	Under conditioned	2
	Emaciated	4
POSTURE	Hunched body posture	1
CLINICAL SIGNS	Tachypnoea (fast breathing)	1
	Dyspnoea (difficulty breathing)	3
	Loose stools or diarrhoea	3
	Hyperthermia	2
	Hypothermia with continued depression	HEP
	Convulsions – intermittent	3
	Convulsions - continuous	HEP
	Ocular or Nasal discharge	3
	Pale extremities	4
ENVIRONMENT	Slightly disorganized nest	1
	Nest barely recognizable	2
	No nest	3
BEHAVIOUR DEPRESSION SCORE	Normal	0
	Mildly depressed	1
	Moderately depressed	2
	Severely depressed	3
	Immobility >24h, very weak, unable to remain upright, comatose	HEP

LOCOMOTION	Slightly abnormal gait/posture, circling	1
	Markedly abnormal gait/posture	2
	Significant mobility problems/reluctance to move	3
PAIN SCORE GRIMACE SCALE	Orbital Tightening	0-2
Not present =0	Nose Bulge	0-2
Moderately visible = 1	Cheek Bulge	0-2
Severe = 2	Ear Position	0-2
	Whisker Change	0-2
HYPERALGESIA	Aggression, isolated, vocalization, guarding	3
TOTAL SCORE		
Additional Observations		

Actions / Interventions to be taken	**Total score**
Review frequency of monitoring	1-7
Increase frequency of monitoring; consider analgesia, supplementary fluids/care nursing care	8-15
Review progress with veterinarian and/or animal welfare specialist	≥15
Implement Humane-endpoint	≥15 with no improvement/progressive illness envisaged

Animals showing any of the following symptoms should be euthanized immediately:

a) Hind limb paralysis which interferes significantly with locomotion, feeding or drinkin;

b) Hypothermia;

c) Nasal discharge with cyanosis;

d) Audible or visible signs of respiratory distress

e) Loss of righting reflex.

ANNEX 4: ANIMAL WELFARE SCORE SHEET: ONCOLOGY STUDY PROTOCOL NUMBER: SHORT TITLE:

Animal ID: Baseline body weight: Max body weight gained:

Humane end point body weight (%)

Date of last treatment/procedure: Monitoring Frequency:

CATEGORIES	INDICATORS	SCORE
DATE		
BODY WEIGHT		
APPEARANCE: BODY WEIGHT	5 % - 10 % weight loss	1
	11 % - 15 % weight loss	2
	16 % - 20 % weight loss	3
	20 % weight loss	HEP
COAT CONDITION	Coat slightly unkempt	1
	Slight piloerection	2
	Marked piloerection	3
BODY FUNCTIONS	Tachypnoea (fast breathing)	1
	Dyspnoea (difficulty breathing)	3
ENVIRONMENT	Loose stools or diarrhoea	1
	Blood in diarrhoea	HEP
BEHAVIOUR	Tense and nervous on handling	2
	Markedly distressed on handling, e.g. shaking, vocalization, aggressive	3
LOCOMOTION	Slightly abnormal gait/posture	1
	Markedly abnormal gait/posture	2
	Significant mobility problems/reluctance to move	3
	Immobility >24h	HEP
PROCEDURE SPECIFIC INDICATOR	Tumour size > 1.2cm – as per AEC approved protocol	HEP
	Tumour ulceration	HEP
	Tumour impeding movement	HEP
	Erythema and swelling of entire paw, including digits	4
TOTAL SCORE		
Additional Observations		

Actions / Interventions to be taken	Total score
Review frequency of monitoring	1
Increase frequency of monitoring; consider supplementary fluids/care or nursing	2
Review progress with veterinarian and/or animal welfare specialist	4
Implement Humane-endpoint	6

ANNEX 5: ANIMAL WELFARE SCORE SHEET: STROKE STUDY PROTOCOL NUMBER: SHORT TITLE:

Animal ID: Baseline body weight: Max body weight gained:

Humane end Point body weight (%)

Date of last treatment/procedure: Monitoring Frequency:

CATEGORIES	INDICATORS	SCORE
DATE		
BODY WEIGHT		
APPEARANCE BODY WEIGHT	5 % - 10 % weight loss	1
	11 % - 15 % weight loss	2
	16 % - 20 % weight loss	3
	20 % weight loss	HEP
COAT CONDITION	Coat slightly unkempt	1
	Slight piloerection	2
	Marked piloerection	3
BEHAVIOUR	Slightly abnormal gait	1
	Markedly abnormal gait	2
	Significant mobility problems	3
	Persistent immobility >24h	HEP
	Tense and nervous on handling	2
	Markedly distressed on handling, e.g. shaking, vocalizing, aggression	3
ENVIRONMENT	Slightly disorganized nest	1
	Nest barely recognizable	2
	No nest	3
NEUROLOGICAL SCORING	Forelimb flexion	1
	Decreased resistance to lateral push (and forelimb flexion) without circling	2
	Same behavior as grade 2 with circling	3
TOTAL SCORE		
Additional Observations		

Actions / Interventions to be taken	Total score
Increase frequency of monitoring; consider supplementary fluids/care	1
Provide supplementary care e.g. extra fluids, wet mash in the cage	4
Review progress with veterinarian/animal welfare manager	5
Implement Humane-endpoint	12

Annex Q
(informative)

Animal welfare incident report forms

Q.1 Purpose

Q.1.1 The animal welfare incident report (see table Q.1) provides a mechanism whereby person(s), who have identified animal welfare incidents related to animal research procedures, treatments, and the care and well-being of laboratory animals, can record important data.

Q.1.2 This data should be recorded, referenced and filed with the relevant Protocol Case History.

Q.1.3 This data may be used as evidence in any animal welfare investigation, allegations of research misconduct, disciplinary hearings or applications for research funding.

Table Q.1 — Example of an animal welfare incident report

<table>
<tr><td colspan="4">1</td></tr>
<tr><td colspan="4">For office use only</td></tr>
<tr><td>Incident report reference No.</td><td>Date received</td><td>AEC protocol No. (if known)</td><td>Principal researcher/researcher

Department</td></tr>
<tr><td colspan="4">Date of incident:</td></tr>
<tr><td colspan="4">Time of incident:</td></tr>
<tr><td colspan="4">Location of incident:</td></tr>
<tr><td colspan="4">Number of animals affected:</td></tr>
<tr><td colspan="4">Animal(s) identification or description:</td></tr>
<tr><td colspan="4">Incident identified by (name, qualifications, contact details and signature):</td></tr>
<tr><td colspan="4">Incident witnessed by (name, qualifications, contact details and signature):</td></tr>
</table>

Incident reported to (date, name, qualifications and contact details):
Description of incident and observations (attach detailed statement if necessary):
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):
Department (if known): **Additional comments or remarks:** **Additional statement(s) attached :** **Yes** **No**
Submit report to the institutional veterinarian or chairperson of the AEC.

Q.2 Checklist for animal welfare incident report

The following list provides key indicators to aid in the completion of the incident report form:

a) animal care and well-being;

b) research animal facility (authorized and controlled access);

c) animal handling;

d) animal monitoring/duty rosters/public holidays/weekends/after hours;

e) biohazards /noxious substances/ionising radiation/chemicals;

f) cage records/case history;

g) caging.

h) emergency procedures/contingency plans.

i) escaped animals;

j) feed and water;

k) Housing;

l) photographic or video evidence;

m) post-surgical procedures or anaesthesia;

n) quarantine animals;

o) relevant and existing SOPs or work instructions;

p) sanitation standards or hygiene standards;

q) staff training and expertise;

r) surgical procedures or anaesthesia;

s) transport facilities or methods/emergency kit; and

t) veterinary care.

Annex R
(informative)

The AEC Templates

R.1 The AEC Application Form

Example of an application form for approval to use animals for scientific purposes.

Submission Date		Project No.	
AEC approval Date		Signature (only on approval)	

a) This form is based on the specifications in the South African National Standard (SANS 10386-2008): ″The Care and Use of Animals for Scientific Purposes″, which defines animal as ″live, non-human vertebrate, including fertilized eggs, fetuses and embryos, i.e. fish, amphibians, reptiles, birds and mammals, and encompassing domestic animals, feral animals, purpose-bred animals, farm animals, wildlife and higher invertebrates such as the *Cephalopoda* and *Decapoda (for example, octopus, squid, cuttlefish)*″.

b) This application should be typed.

c) It should be signed by the Principal Investigator (the applicant) and other persons who are vouching for specialised aspects of the experimental design (for example, statistician, safety officer, and persons responsible for supervising the use of scheduled medicinal substances) as indicated.

d) Applications that have not been signed will not be considered.

e) All applications need to have an assigned veterinarian for emergency management of animals and to make a decision on study termination per chance a welfare concern arises .

f) The application needs to be written simply, briefly and is not to exceed the limitations indicated.

g) The application should be submitted electronically as well a hard copy.

h) The monthly deadline for submissions is two weeks prior a meeting.

i) The date at which the completed application is submitted on line offices will determine when the application will be considered.

j) To meet the requirements of SANS 10386, a progress report will be requested annually.

A: PROJECT TITLE

B: PRINCIPAL INVESTIGATOR/RESEARCHER *(Corresponding author*

Yes		*No*	

Name	Contact Number	e-mail address	Contact Address

Qualifications & student number	
Appropriate experience in animal research	
Professional Registration/Authorisation number (e.g. SAVC/HPCSA)	

C: SUPERVISOR/CORRESPONDING AUTHOR (if applicable) *(Corresponding author*

Yes		*No*	

Name	Contact Number	e-mail address	Contact Address

Student number	
Qualifications	
Appropriate experience in animal research	
Professional Registration/Authorisation number (e.g. SAVC/HPCSA)	

D: Co-WORKERS (involved directly with procedures on Animals)

Name	Contact Number	e-mail address	Contact Address

Qualifications	
Appropriate experience in animal research	

Name	Contact Number	e-mail address	Contact Address

Qualifications	
Appropriate experience in animal research	
Professional Registration/Authorisation number (e.g. SAVC/HPCSA)	

E. DECLARATION

1. Moral Philosophy

The ethical review of proposed animal experiments is predicated upon the acceptance that non-human animals are organisms fully worthy of moral concern and as such their interests must be protected as far as possible in their use for advancement of biological knowledge and for the promotion of the health and welfare of animals and humans and protection of the environment.

2. Animal Interests

In the use of laboratory animals, animal interests obligate scientists and educators to:
not allow animals to be used for research and/or to be killed for trivial, irrational, unjustified or inappropriate reasons;
permit animals to live, reproduce and grow under conditions that are comfortable and reasonably natural to their species;
keep animals free from disease, parasitism, injury and pain by prevention, rapid diagnosis and treatment;
allow animals to be able to express normal behaviour through providing as far as possible sufficient space, proper facilities in which to live and in the company of the animal's own kind recognising the inherent social nature and hence the necessity of a social relationship for many species;
protect animals from fear, deprivation, stress, distress and pain by ensuring that their living conditions, handling and treatment will be such that it will either minimize or eliminate the causation of these states upon those animals, that are used for research, teaching and testing;

3. Humaness

The principles of humane experimental technique proposed by Russell & Burch must be followed in the planning and conduct of animal experiments. These comprise:
Replacement of animals with non-sentient research systems, i.e. researchers must strive to avoid using of laboratory animals if alternative methods can yield the data they need.
Reduction of the numbers of animals that should be used to a minimum by design in order to achieve only sufficient statistical power to allow the objects of the experiment to be achieved.
Refinement of the experimental methodology to be adopted by the implementation and if necessary by the improvisation of procedures which will have the least distressing or harmful effect to the animals and when this is not avoidable to counter those effects by the use of ataractics (tranquillisers), neuroleptics (dissociative agents), anaesthetics, analgesics and other effective strategies.

4. Animal Protection

Animals should be protected from research designs that involve pain, illness, isolation, mutilation (whether by surgery or otherwise) and/or premature death until such research can be demonstrated to be absolutely imperative and related to health, welfare and environmental problems, which are potentially catastrophic in nature and for which alternative designs using non sentient systems are not feasible.

5. Relevance

Animal based teaching and research must address an important question relevant to the objectives in advancing knowledge, education, science and human and animal welfare through research, be based on plausible hypothesis and have a reasonable prospect of yielding good results.

6. Responsibility

It is the responsibility of everyone using animals, for experimentation, testing diagnosis, teaching, sourcing of tissues or body fluids to assure that the animals that they use are afforded the highest levels of welfare and protection from abuse.

7. Policies

This committee has a number of policies in place that may be relevant to the application. Your attention is specifically drawn to:

SANS 10386, "The care and use of animals for scientific purposes", which defines the responsibilities.

Terms of Reference of this committee.

Institutional policies.

8. Personal Declaration

I/We, (full name) ……..……..., as Principal Investigator or and ……..……..., as Supervisor in this application, hereby declare that we are familiar with the precepts, policies and responsibilities outlined under Section E and will personally undertake to ensure that these are upheld in the conduct of this study, should it be approved. I/We will ensure that this research conforms to the applicable legal requirements.

I/We undertake not to deviate from the approved application without obtaining prior approval by the AEC for any desirable or necessary significant changes that may need to be made in the methods used, which may affect the welfare of the animal subjects.

……..…… ……

Signature of Principal Investigator **Date**

In my opinion, all persons named and working under my supervision have the appropriate training and skills needed to carry out their responsibilities for experimental procedures, care and handling of the species being used.

…… ……

Signature of Supervisor(When applicable) **Date**

F: SCIENTIFIC REVIEW STATEMENT

(Every application should be supported by a declaration that it has undergone prior scientific review outside of the applicant's Unit or Group. **One** of the following 3 statements must be completed.)

This research protocol has been reviewed by a scientific review committee …….. ……..(please specify) of the (Institute/Unit) …… …… on (date) ……..…… and has been judged to be relevant, designed in accordance with accepted scientific practices and norms and is in the opinion of the reviewers likely to be successful in achieving its objective.

…….. ……

……..……

Name and Designation	**Signature**
Date	

OR

Funding for this research project has been received from ……..…….. (agency) that requires a review process prior to funding research projects.
Grant holder:
……
……..
Type of grant: ……..……..Grant number:
……..

…….. ……..……
……..……
Grant holder **Signature**
Date

OR

I, in my capacity as Head of Department ……..……, confirm that this application has been judged to be relevant, designed in accordance with accepted scientific practices and norms and in my opinion is likely to be successful in achieving its objective.

…….. ……..……
……
HOD **Signature**
Date

G: FUNDING

Is this project fully funded	**YES**	**NO**
Does the funding of the project depend on the project being approved by the Ethics Committee?	**YES**	**NO**

H: TYPE OF RESEARCH

Academic		Contract		For degree purposes		Indicate Degree	

Research Category : PAIN/DISCOMFORT/STRESS CLASSIFICATION					
A1	Experiments on embryonated eggs or cephalopods and decapods' or stored specimens previously collected from animals	□	A2	Studies on vertebrate animals during the course of routine examination, teaching procedures and treatment. **Examples:** *Animals held under proper conditions for later use or for teaching non-invasive procedures.* *Observational studies e.g. on wild animals in the field.*	□
B	Procedures on vertebrate species that are expected to produce stress but no pain requiring anaesthesia. **Examples:** *Wild animals caught in the field, caged and transported for observation.* *Administration of medication.* *Collection of blood specimens and swabs.* *Rectal examination*	□	C	Experiments that produce minor or short-duration pain requiring the use of pain relieving drugs. **Examples:** *Subcutaneous implants.* *Docking in sheep.* *Collection of tissues from animals after euthanasia.*	□
D	Experiments that involve significant but unavoidable stress or pain requiring anaesthesia or a humane endpoint.	□	E	Procedures that involve inflicting severe pain at or above the pain tolerance threshold and the use of pain.	□

	Examples: *Non-survival surgery in teaching.* *Survival surgical procedures.* *Infectivity or toxicity studies with a high probability of producing disease*. *Large animals kept in boma*				relievers are contra-indicated. **Examples:** *Toxicity/virulence testing where death is the endpoint.* *Disease/cancer models involving chronic clinical signs.*	

**Definitions from Special Issue, Laboratory Animal Science, January 1987, p 12*

I: PROJECT

1. Commencement of research

Expected Starting Date		Expected Completion Date	
I declare that the project has not commenced without approval (signature)			

2. Brief justification

(Provide a brief introductory statement NOT EXCEEDING 500 WORDS and *supported by relevant scientific literature* that explains what problems, questions, needs or scientific or clinical observations or new ideas have led to the planning of the experiment.) *(Please type)*

3. Aim(s) of the proposed study

(State these briefly and succinctly.) *(Please type)*

4. Potential benefits of the research findings

(These are required to aid the reviewing committee in performing a harm/benefit assessment.) *(Please type)*

5. Hypothesis

(If a hypothesis is being tested give the postulate/s (null hypothesis and alternates) to aid the reviewers in following the rationale of the proposed study.) *(Please type)*

6 Animal requirements

Animal Species *(Please state whether domesticated or not)*							
Strain				Total Number Required			
Gender: Male		Female		Body mass		Age	
Microbial Status				Source of Animals			

7. Specimens derived from Animals

Sample Type	Number	Volume	Species	Previous AEC approval	Location/Country
e.g. Serum	*200*	*1ml each*	*Leopard*	*Protocol nr*	*Biobank*

For studies making use of only specimens previously collected from animals: Sections 10, 12, 18, 23, and 24 must be completed

8. Justification for the use of sentient animals

(Briefly justify the use of animals, the choice of species, the numbers to be used. If there is limited availability, or large numbers should be used, provide additional rationale for their selection and numbers. State also what non-sentient model/s or non-animal models were considered and on what grounds they were rejected.) *(Please type),*

9. Reduction of the number of animals to a minimum to achieve scientific objectives

(Describe how this was determined either by calculation (statistical design) or by specification (i.e. use of a validated testing protocol) or any other strategy.) *(Please type)*

10. Animal housing and care

(Briefly describe how the animals will be housed (penned, stabled, caged or confined in any other way, kept in metabolic crates or cages, etc.), their nutrition (feeding and watering) and what provisions have been made for the physical and psychological well-being i.e. comfort, socialisation, behavioural needs and enrichment of their immediate environment.) *(Please type)*

11. Facility Details

NAME OF FACILITY USED:	
SAVC Registration Number (For animal research facilities or Veterinary Laboratory facilities)	
PHYSICAL ADDRESS	
EMERGENCY CONTACT NUMBER	
AUTHORISED SIGNATURE & DATE:	

12. Statement of animal care competence, expertise and experience

(Provide a short statement of the scientific knowledge competence and experience of the person(s) appointed to ensure the comfort, health and humane treatment of the animal subjects in this study) and provide their registration credentials either with the South African Veterinary Council, the Health Professions Council of South Africa or the South African Council for Natural Sciences Professions, and any in-house accreditation obtained.) *(Please type)*

13. Experimental design

(For all studies submitted to the AEC: Explain the reasoning behind the study design and experimental planning, with particular reference to determination of sample size and statistical analysis. The use of flow charts is recommended. The information should be presented in an easily accessible manner. For studies involving animals also, describe how the animals will be allocated to experimental and control groups and where applicable, how the experimental treatments will be assigned to each group.) *(Please type)*

14. Restraint of the animals

(Describe the methods of physical (manual procedures and use of special restraint equipment) or chemical restraint to be used on the animals and state who the animal handler/s will be.) *(Please type)*

15. Experimental animal procedures

(Describe briefly in short annotated sentences IN SEQUENCE, all the steps that will be performed in conducting the proposed experiment. These include: duration of animal holding and animal use, the collection of specimens (if body fluids give routes of collection and volumes), operative procedures, etc.) *(Please type)*

16. Administration of all medicines/substances

(List ***all*** substance administrations to the animals and give routes of administration, dosages per body mass including anaesthetics, analgesics and euthanasing agents. State who is legally responsible for prescribing and directing the administration of the controlled Scheduled 3 – 6 medicinal substances and other controlled substances and provide their acceptance of this responsibility by signature. **Please note that it is expected that animals experiencing painful conditions will be given appropriate analgesic and/or anaesthetic suppor**t) *(Please type)*

Responsible person (print name)	
Qualification	
Acceptance of responsibility: **SIGNATURE & DATE:**	

17. Severity of effects of the experimental procedures on the animals

(List the procedures that may cause deprivation, fear, distress and pain. Describe what sensations the animal may feel. Categorise these as minimal, intermediate or high. *Give their likely duration in time. Describe what specific steps will be taken to alleviate these conditions through the use of ataractics, dissociative agents, analgesics, anaesthetics or other methods. Estimate how effective these are likely to be.) *(Please type)*

* *Laboratory Animals 24: 97 – 130, 1990).*

18. Fate of animals and their disposal at the end of the study

(Briefly state the *fate* of the experimental animals at the end of the study, for example, rehabilitation and release, return to stock, euthanasia; released into its natural environment). What method of euthanasia is to be used, what humane rationale supports this choice and how the animals or animal carcasses should be disposed of in a responsible and ecologically sound manner.) *(Please type)*

19. Statistical analysis

(Describe briefly how the data obtained from the study will be analysed statistically, explain this decision and state by whom the analyses will be performed.) *(Please type)*

20. Refinement

(Describe the specific steps that have been taken to refine the experimental procedures to make them as humane as possible i.e. minimising the impact of the proposed procedures on the animals' well-being e.g. use of enrichment aids, analgesia, etc) *(Please type)*

21. Monitoring of experimental animals

(Describe who will be responsible for the care of the animals during the experimental period, and provide an indication of their experience and competence. Briefly state what clinical, physiological or behavioural criteria (or both) will be specifically monitored to assess the animal's well-being e,g. weight gain/loss, food intake, vital parameters, etc. Please note that any study that has the potential to interfere with growth or cause weight loss, will need a minimum of weekly weight monitoring) *(Please type)*

22. End points for experiments in animals

(Provide the points at which this study will be terminated for welfare reasons e.g. percentage weight loss, injury, animals showing distress, pain, animals becoming moribund. Also provide how the monitoring towards these endpoints will be undertaken, for example, . weekly weights, twice daily observations) *(Please type)*

23. General veterinary care

(Please note that this section is compulsory. Provide details, including emergency contact details, of the veterinarian who will be responsible to provide the general veterinary care and who will have the authority to enforce the endpoints stipulated under Point 20. The veterinarian must be registered or authorised by the SAVC and is preferably independent of the research group.) *(Please type)* **It is the responsibility of the veterinarian to arrange for a locum if he/she is not available.**

PERSON RESPONSIBLE FOR VETERINARY CARE OF ANIMALS				
EMERGENCY Contact details				
Veterinarian resident at facility (Tick)	Resident		Non-resident	
Schedule of visits if not resident at study or research site (e.g. weekly; for emergencies only; only available telephonically) and for off-site studies the distance of the study to the veterinarian				
Management of injured animals (Please provide the management practices for minor and/or major injuries/disease occurrence.)				
Signature & Date				

24. Personnel activities

(Describe the specific *responsibilities* and *duties* of EACH PERSON who will be involved with the procedures on animals, preferably in a tabular format.) *(Please type)*

25. Biohazard statement

(Does the project pose any hazards to other animals and/or staff from the use of infective agents, toxic substances, carcinogenic agents or ionising radiation? If it does, state the specific safety procedures to be followed to contain these hazards and provide an approval statement in the space below from the Institutional Safety Officer. If available, you may append the laboratory's relevant SOPs and policies.) *(Please type)*

FACULTY:	
NAME of OFFICER	
SIGNATURE & DATE:	

26. Declaration for studies needing external approval

(Please provide a list of external approval that this following project requires e.g. section 20 approval from the relevant national department (see foreword); Section 21 approval from the MCC for any studies involving an unregistered medicine; TOPS approval from working with an Threatened and Endangered species; Approval from the relevant Nature Conservation organization and/or Provincial Authority to work with wild species)

R.2 AEC Annual Progress or Final Report Form

Animal Ethics Committee (AEC)

ANNUAL PROGRESS ☐

or

FINAL REPORT FORM ☐

Please tick appropriate box

	For office use only
NOTE: Principal Investigators must ensure that they submit an Annual report for all open AEC protocols, and a Final report for completed protocols, by the 28th of February for each preceding year. Failure to comply with this requirement will result in the study being suspended, or other action being taken by the AEC. **No sections of this form may be changed or removed, other than expanding available space in the text blocks provided.** **All sections of this form must be completed.** **This form must be typed and submitted to:** **Phone:** **E-mail:**	AEC reference number: Study severity category: Study expiration date: Date report received: Date report evaluated: **Status of protocol:** Open Completed Discontinued (closed) **AEC decision (open studies):** Study to continue Modifications required Study to be suspended Study to be terminated

Name of Principal Investigator	
Department / Division / Unit	
Protocol reference number	
Protocol title	
Number and species approved	
Number of animals used to date	
Date of study commencement	

Envisaged date of completion	
Describe the status of the protocol, i.e. whether it is to continue, has been completed, or is discontinued (i.e. closed)	
Briefly outline the progress achieved in the study to date	
Briefly outline the likelihood of the study or teaching activity achieving the stated objectives	
Briefly outline any problems that may have interfered with the progress of the study	
Briefly report on the well-being and welfare status of animals, including any unanticipated problems, in the previous year	
Number of animals that died during procedures, and reasons for death, in the previous year	
Number of animals that died at all other times, and reasons for death, in the previous year	
Number of animals that were euthanized, due to humane endpoints reached, in the previous year	
Number of animals that were euthanized, due to unexpected reasons, in the previous year (describe reasons)	
Briefly outline envisaged future amendments (i.e. modifications or additions) to the protocol	
List any new publications arising from the study, or those that are under preparation	
Signature of Principal Investigator	
Date	

For office use only: Report considered by the FHS AEC

Date report evaluated by the AEC:

Status of protocol:

- ☐ Open
- ☐ Completed
- ☐ Discontinued (closed)

Outcome of AEC decision (for open protocols):

- ☐ Study to continue
- ☐ Study modifications required
- ☐ Study to be suspended
- ☐ Study to be terminated

Signature (AEC Chair):

Comments:

Annex S
(informative)

Embryo and stem cell research

S.1 General

Preimplantation embryos are the very early forms of life after an oocyte and sperm have fused until implantation in the uterus of the female. This period of development takes approximately 4,5 days in mice and 7 days in bovine.

S.2 Growth stages of a pre-implantation embryo

S.2.1 Embryos are covered in a protein "shell" called the zona pellucida which protects the embryo and prevents it from attaching to the oviduct wall before it reaches the uterus. The single celled zygote (fertilized oocyte) divides into two cells, then four, then eight and so on. This is the cleavage stage. Cell divisions in normal embryos are always even in number and the cells or blastomeres are also even in size, at each division.

S.2.2 When an embryo reaches 8 cells to 16 cells, the blastomeres start to become compacted (flatten up against each other) and the embryo changes to form a mulberry-like structure called a morula.

S.2.3 At approximately the 32 cell stage some blastomeres begin to pump fluid into the intercellular spaces. A cavity of coalesced fluid forms of gradually increasing size which pushes the blastomeres outwards to press against the zona pellucida. The embryo is now called a blastocyst. The clustered pluripotent cells which collect to one side is called the inner cell mass (this forms the embryo proper) and the epithelial outer cells form the trophectoderm (this gives rise to extraembryonic tissues like the placenta). The blastocyst cells continue to divide and collect fluid causing the zona pellucida to thin out, weaken and tear open allowing the blastocyst to ″hatch″ out and implant in the uterine wall.

Table S.1 — Comparison of embryo development stages recorded as the approximate number of days after fertilization for mice and bovine

1	2	3
Stage	**Mouse**	**Bovine**
Zygote	0,5	1,0
2-cell	1,5	2,0
4-cell	2,0	3,0
8-cell	2,5	3,5
Morula	3,0	5,0
Early Blastocyst	3,5	6,0
Late Blastocyst	4,5	7,0

S.3 Methods of embryo production

S.3.1 Embryos from animals can be obtained by the following three methods (but not limited to):

a) In vitro fertilization (IVF) – oocytes are obtained from a donor female after super-ovulation and combined with sperm from a donor male to fertilize in the laboratory; fresh or cryopreserved sperm may be used. Zygotes are cultured to the desired embryo stage for further manipulation.

b) Embryo Flushing – superovulation: An embryo donor is super-ovulated and mated as per normal. Embryos are then flushed from the oviducts at the desired growth stage and collected for further manipulation.

c) Embryo Flushing – natural ovulation: An embryo donor is monitored for the correct oestrus cycle. Once in proestrus the female is mated as per normal. Embryos are then flushed from the oviducts at the desired growth stage and collected for further manipulation.

S.3.2 Superovulation requires the use of hormones such as Pregnant Mares Serum Gonadotropin (PMSG) and Human Chorionic Gonadotropin (HCG) in order to synchronize ovulation and maximize the number of oocytes produced. However, in some strains or breeds this type of stimulation may cause reduced quality embryos, therefore, natural ovulation would be considered. Embryo quality should always precede embryo quantity.

S.4 Embryo culture and consumables

S.4.1 Embryos and gametes are manipulated while in culture media. Many companies supply different media types which are often made to support a specific stage of embryo development. Media for handling embryos in ambient air when outside of the incubator should contain pH buffers such as HEPES or MOPS (at 5 % to 7 % carbon dioxide) typically use bicarbonate buffers. Media may contain foetal bovine serum or bovine serum albumin and importation may require a permit from the relevant national department (see foreword) permit.

S.4.2 Embryos can be single cultured or co-cultured in tissue culture dishes or 4-well dishes. To prevent evaporation, temperature loss and changes in osmolality media can be covered in sterile filtered mineral oil or liquid paraffin. It is recommended that culture medium is renewed every 48 h in order to provide growth stage-specific nutrients and prevent toxicity. All procedures performed in the laboratory should follow aseptic technique.

S.4.3 All culture dishes, test tubes, etc. should be clearly and permanently labelled with a unique identification so as to easily identify the sample and date of preparation.

S.4.4 All culture media and disposables should be labelled with the date received, date opened and expiry date. All consumables and media should be suitable for embryo culture and fit for their purpose. Sterile single-use disposable consumables and the use of quality controlled media and oil is recommended. Packaging integrity and appropriate delivery conditions should be checked. Products and materials used should be traceable.

S.5 Optimal culture conditions

S.5.1 Microenvironment

S.5.1.1 Mammalian preimplantation embryos normally develop in the protective environment of the female's reproductive tract. In research, we should attempt to simulate this environment. In order to optimize embryo development, adequate conditions of temperature and pH should be maintained to minimize fluctuations of culture conditions and protect embryo homeostasis.

S.5.1.2 Embryos are routinely cultured in large carbon dioxide (CO_2) incubators which use medical or high grade technical CO_2 gas in order to maintain the correct pH in media. Smaller benchtop incubators which use premixed tri-gas, a combination of CO_2 and oxygen (O) balanced with nitrogen may also be used and are advised for more sensitive species.

S.5.1.3 Incubators should be connected to emergency power back-up. The incubator should be monitored daily for the correct temperature and carbon dioxide content before the first door opening. This should be done using calibrated independent temperature and gas detection methods, not by digital display alone.

S.5.1.4 Incubators should be set to a temperature reflecting the body temperature of the animal embryos to be cultured, 37 ˚C for mice or 38,5 °C for bovine. Incubators should be humidified to approximately 90 % to 95 % to prevent evaporation of media and a shift in osmolality.

S.5.2 Physical environment

S.5.2.1 The laboratory environment in which embryos are cultured plays a large role in the success and quality of embryos cultured.

S.5.2.2 The laboratory should be in a low traffic secure area, preferably isolated from other laboratory functions by a physical non-porous wall. The walls, floors, benchtops etc. should be constructed of material which contains low volatile organic compounds (VOCs) and is easy to wash and disinfect with the appropriate non-toxic products.

S.5.2.3 The laboratory should be supplied with a heating, ventilation and air-conditioning (HVAC) system which supplies high efficiency particulate air (HEPA) (high efficiency particulate air) filtered air, preferably combined with a photo-catalytic oxidising system or charcoal filter. Environmental toxins can accumulate in culture media and may adversely affect embryo development, this combination of filters will reduce the amount of VOCs, heavy metals and particles to provide a pathogen free, non-toxic air environment. Seven to fifteen air changes per hour are recommended.

S.5.2.4 It is recommended that the laboratory be lit with ceiling mounted, dimmable (200 lux – 400 lux), longer wavelength (>500 nm) warm light. Blue light (400 nm to 500 nm) appears to be more harmful to developing embryos, the extent of damage relating to exposure time, wavelength and intensity.

S.5.2.5 The temperature of the laboratory should be set at 21 °C to 25 °C with a relative humidity of 30 % to 50 %, or as indicated appropriate by scientific evidence. Consistency of conditions is key to repeatable success. Variations in results are evident when conditions are not consistent. Drafts in the laboratory should be avoided at all costs.

S.5.2.6 Appropriate refrigeration facilities should be available for storage of media and reagents. Avoid repeated shifts of temperature while handling in the laboratory.

S.5.2.7 A separate office space should be provided for data capturing, filing, reference books, journals and other administrative work.

S.5.2.8 Eating, drinking, smoking, application of make-up and manipulation of contact lenses are not permitted in the laboratory. Perfumes and other strong smelling personal products should be avoided.

S.5.2.9 The use of toxic chemicals (such as toxic cleaning materials), aerosols and pest control is not permitted. Where possible the formation of aerosols and droplets should be avoided in the laboratory.

S.5.2.10 An area for cleaning and equipment sterilization, the use of fixatives and toxic reagents should be available separate from where embryos and gametes are handled.

S.5.3 Embryo handling and equipment

S.5.3.1 Handling of embryos should occur at constant temperatures, for example, the same temperature in the incubator or on the workstation surface. This can be achieved by making use of water baths, slide warmers, heating blocks, heated workstation surfaces, etc. Fluctuations in temperature are detrimental to embryos and gametes.

S.5.3.2 A preimplantation embryo remains relatively the same size from the 1-cell stage to the early blastocyst stage before increasing in size prior to hatching. It is, therefore, necessary to use the correct equipment to properly handle and observe the embryos during the specific cell stages. Typical equipment used includes:

a) Pipettes: Mechanical pipetting devices should be used wherever possible for the manipulation of liquids, gametes and embryos. Manufactured plastic and glass micropipette tips are available for the different pipetting devices. These can be purchased in various tip diameters, depending on the embryo development stage and embryo size. Some laboratories may prefer to use glass pipettes with a non-toxic rubber or silicon bulb so that they may pull their own pipettes to create unique tip diameters.

b) Microscopes: In order to visualise and manipulate embryos in a culture dish a microscope is used. Stereo microscopes provide an adequate space for manipulation on the microscope stage at a lower magnification range. Many routine procedures are performed on stereo microscopes and so they are an essential tool in the laboratory. Inverted microscopes provide a higher magnification as well as manipulation space on the stage for embryo culture dishes. Inverted microscopes are preferred for up close imaging, embryo scoring and micromanipulations. These microscopes will need to accommodate specialised illumination techniques such as contrast enhancement. Upright bright field microscopes with phase contrast objectives are used to observe semen specimens and 100X immersion objectives are used to assess stained morphology slides.

c) Laminar flow benches or biological safety cabinets (BSCs): These types of cabinets act as the primary protection barriers when handling biological materials. Depending on the type of cabinet they either protect the material being manipulated within the hood from worker or environmental sources of contamination (horizontal laminar flow cabinet), or they protect the laboratory worker and laboratory environment from exposure to infectious or other hazardous materials that are present within the hood (BSC Type II). Both types of hoods use a HEPA filter with blowers that generate a non-mixing stream of air.

S.5.3.3 Equipment should be maintained and calibrated as appropriate for the type of equipment. Functional checks and calibrations should be recorded on a daily, monthly or yearly frequency (as appropriate).

S.6 Embryo scoring

S.6.1 Embryo scoring assesses the quality of an embryo as it grows. These observations provide an idea of which embryos are healthy and are likely to implant versus which are unhealthy and may arrest in development. Embryo scoring takes into account the following factors:

a) The timing of cell divisions – the best score will be given to divisions that occur on time. Too slow and too fast are both undesirable traits.

b) The number of cells after each division – cell numbers should be even up until the 16-cell stage. Uneven numbers of cells is undesirable.

c) The morphology or appearance of the cells, for example, size, shape, colour, plumpness, vacuoles, multinucleation. Cells should be plump, even in size, without vacuoles. Cells ″deflate″ and take on a brownish colour when they begin to degenerate.

d) The degree of fragmentation – small fragments of cells sometimes remain in the perivitelline space after a cell division. Large percentages of fragmentation in an embryo are undesirable.

S.6.2 Blastocyst grading uses a different system entirely. For blastocysts the scoring is broken down into

a) the volume of the blastocoel (fluid cavity) in relation to the volume of the embryo,

b) the thickness of the zona pellucida,

c) the number and the degree of compactness of the cells in the inner cell mass,

d) the number and degree of tightly knitted cells in the trophectoderm, and

e) the degree of hatching versus. a fully hatched blastocyst (completely out of the zona pellucida).

S.6.3 Many embryo scoring systems exist and are well described in the literature. Each technician or researcher in a laboratory should learn and use the same method of scoring in order to standardise their observations and results. (Nasiri N & Eftekhari-Yazdi P, 2015).

S.7 Embryo cryopreservation and storage

S.7.1 Embryos and gametes can be cryopreserved (frozen) and stored in liquid nitrogen for long periods of time. Two main styles of cryopreservation exist, controlled-rate cryopreservation and vitrification. For sperm, controlled rate freezing is preferred. Embryos and oocytes can be frozen by either method.

S.7.2 Cryopreservation can cause damage to the embryos and gametes, therefore, media should contain a cryoprotectant and only the best specimens should be considered for cryopreservation. Discarded embryos are disposed of as biological waste.

S.7.3 Embryos and sperm can be stored in either open or closed systems, in plastic straws or cryo vials or specialized vitrification devices.

S.7.4 At the time of cryopreservation be sure to record the date and time of cryopreservation, cryopreservation method and media used, embryo quality and stage of development, the number or volume of embryos or gametes per device, the number of devices stored, the location in the cryostorage tank, the operator. Labels on the devices should be permanent and able to withstand the stressors of remaining at very low temperatures (-196 ˚C) without rubbing off, becoming brittle or breaking.

S.7.5 Safety issues have been raised regarding the direct contact of biological material with liquid nitrogen as cross-contamination of infectious pathogens is possible. It is therefore optional to store specimens under liquid nitrogen or in vapour phase tanks. Regardless of the type of storage, care should be taken when working in the tanks as temperatures should never rise above -130 °C.

S.7.6 The correct personal protective equipment should be used when working with liquid nitrogen. Personal low oxygen alarms are recommended as an additional safety measure (see S.11).

S.7.7 Cryopreservation facilities should be separate but near to the laboratory, preferably with visible access to the interior, for example, via a window, and good ventilation. Cryostorage units should be routinely monitored for levels of liquid nitrogen or out of range temperatures. Alarm systems may be fitted.

S.7.8 For security purposes, liquid nitrogen dewars should be securely locked after use. Samples should preferably be stored in two different locations.

S.8 Transport

S.8.1 Cryopreserved sperm specimens may be transported in their storage vials or straws on dry ice. The storage vessel should be in direct contact with equal amounts of dry ice on all sides to maintain a stable temperature.

S.8.2 The preferred method of transportation of cryopreserved gametes and embryos is using a dry shipper which is able to maintain very low temperatures. Dry shippers contain absorbent material which can be saturated with liquid nitrogen; they do not contain free liquid nitrogen. This allows for more flexibility when shipping non-hazardous cryopreserved materials.

S.9 Hygiene

The following are examples of good laboratory hygiene, which includes but is not limited to

a) use of low particle-shedding protective laboratory clothing,

b) use of hairnets and non-toxic, non-powdered gloves and facemasks where appropriate,

c) use of appropriate laminar flow benches or biological safety cabinets for handling biological materials,

d) use of aseptic technique in which non-contaminating conditions should be maintained,

e) use of specific disinfectants with proven compatibility and efficacy for an IVF laboratory,

f) single-use consumables correctly and immediately disposed of into proper waste containers. Potentially infectious materials should be disposed of in accordance with biosafety policies that protects laboratory and other staff from exposure, and

g) minimal or unavoidable use of needles, glassware and other sharps which should be handled with extreme caution and discarded into sharps containers.

S.10 Record keeping

S.10.1 Each process in the laboratory should have a written SOP.

S.10.2 Each sample should have complete traceability with records maintained accordingly.

S.10.3 All media, reagents, disposables etc. should be tested for quality using appropriate assays whenever possible.

S.10.4 Service records, function checks and calibrations for all equipment should be recorded and accessible.

S.10.5 An emergency plan that aims to ensure the safety of personnel, protect the fresh and cryopreserved animal material and limit damage to equipment and research records should be in place.

S.11 Health hazards

S.11.1 Zoonosis: Zoonotic diseases are diseases which spread between animals and humans. Zoonotic diseases can be caused by viruses, bacteria, parasites and fungi which could be transmitted by coming into contact with the saliva, blood, urine, or faeces of an infected animal. Care should be taken when working with animals which may pose a risk.

S.11.2 Most, if not all embryo culture laboratories will work with carbon dioxide gas and liquid nitrogen. These items should always be kept in an area with good ventilation. Gas detection monitors should be in the vicinity of liquid nitrogen storage vessels or carbon dioxide gas bottles to ensure the area is safe to enter. The appropriate personal protective equipment (PPE) should be worn when working with these items.

S.11.3 Carbon dioxide: Carbon dioxide at levels of 10 % or above can act as a simple asphyxiate by diluting the concentration of oxygen in air below the levels necessary to support life. Carbon dioxide is heavier than air and will tend to concentrate at lower levels. Gas bottles should be located outside the laboratory, should be stacked vertically and should be secured to a wall by a chain at all times to prevent falling over. After connecting a gas bottle to a gas line or manifold, check the connection for any leaks. Carbon dioxide gas levels should be monitored on a daily basis.

S.11.4 Liquid Nitrogen: Liquid nitrogen is a colourless odourless cryogenic liquid. The liquid nitrogen to gas expansion ratio is 1:694 at 20 °C. It is a hazard as release of its gas may cause asphyxiation by rapid displacement of oxygen. The gas is liquefied at -196 °C and can therefore cause freezing of tissue, cryogenic burns and symptoms similar to frostbite upon contact. Check canisters for leaks by the presence of ″sweating″ on the external surface.

Annex T
(informative)

Education, training and competence of personnel

T.1 The education and training of personnel who use and care for animals, is described in terms of a recommended modular training structure, with associated learning outcomes and assessment criteria, in the following reference, as an example of the current best practice:

European Commission. National competent authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes. A working document on the development of a common education and training framework to the requirements under the Directive. Brussels, 2014.
Available from: http://ec.europa.eu/environment/chemicals/lab_animals/pdf/Endorsed_E-T.pdf

T.2 The training structure should include reference to the South African regulations and South African National Standards (SANS), as relevant.

T.3 It is appreciated that it may be difficult for small establishments to meet these standards, so that these are referenced here as an example of best practice recommendations to be strived for.

T.4 The requirement to complete a given module in this training structure is determined by which functions the trainee will perform, i.e.

a) carrying out procedures on animals (including capture and restraint),

b) designing procedures or projects (or both);,

c) taking care of animals, or

d) killing animals.

T.5 The learning outcomes deal with output rather than processes and help to define the skills and knowledge that participants should be able to demonstrate by the time these learning outcomes are assessed. Learning outcomes are the specific intentions of a training programme or module. They describe what a student should know, understand, or be able to do at the end of that module. These, therefore, do not represent a course syllabus or a list of topics to be covered.

T.6 The framework has been designed as a guideline in order to allow delivery of the learning outcomes for each module in a manner that meets the national and institutional requirements.

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