

Guidelines on the Care and Use of Animals for Scientific Purposes

Second Edition

2022

National Advisory Committee for Laboratory Animal Research (NACLAR)

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Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (8th edition, 2013)

National Health and Medical Research Council, Australia

Good Practice Guide for the Use of Animals in Research, Testing and Teaching (2019)

National Animal Ethics Advisory Committee, New Zealand

Guide to the Care and Use of Experimental Animals, Volume 1 (2nd edition, 2017)

Canadian Council on Animal Care (CCAC), Canada

Guide for the Care and Use of Laboratory Animals (8th edition, 2011)

National Research Council, USA

Institutional Animal Care and Use Committee Guidebook (2nd edition, 2002)

Applied Research Ethics National Association (ARENA)/ Office of Laboratory Animal Welfare (OLAW), National Institute of Health, USA

Public Health Service Policy on Humane Care and Use of Laboratory Animals (2015)

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PREFACE TO THE SECOND EDITION

The *Guidelines on the Care and Use of Animals for Scientific Purposes*, first published in 2004 by the National Advisory Committee for Laboratory Animal Research (NACLAR) is a national guide establishing the best practices setting out the responsibilities of all the parties involved in the care and use of animals for scientific purposes in accordance with widely accepted scientific, ethical and legal principles. As stipulated by the NACLAR *Guidelines*, all proposed use of animals for scientific purposes in an institution must be evaluated and monitored by an Institutional Animal Care and Use Committee (IACUC).

Fundamental to the NACLAR *Guidelines* is the 3Rs Principle of *Replacement*, *Reduction* and *Refinement* first introduced by William Russell and Rex Burch in 1959: to *Replace* the need for animal use by alternative means; to *Reduce* the numbers of animals used to an unavoidable minimum, and to *Refine* any procedures necessarily used, so as to minimise the impact on animals, consistent with the achievement of a justifiable scientific purpose.

The first edition of the NACLAR *Guidelines* acknowledged the best practices of countries such as Canada and the USA, and organisations such as the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986). We have continued to take guidance from these and other established authorities to ensure that the *Guidelines* is up-to-date and consistent with widely accepted best practices.

The three main sections of the *Guidelines* issued in 2004 have now been joined by a fourth section on *Occupational Health and Safety*. The four sections, which should be read together as a complete document, are:

“*Guiding Principles*”, which describes the overall guiding principles to promote the humane and responsible care and use of animals for scientific purposes in Singapore, in accordance with the 3Rs. Its scope covers all aspects of the care and use of animals for scientific purposes including their use in teaching, field trials, environmental studies, research, diagnosis, product testing, and the production of biological products, and outlines the responsibilities of institutions, principal investigators and persons involved. All research facilities which house and use animals for scientific purposes have to operate in accordance with the *Guidelines* to qualify for licensing from the Animal and Veterinary Service (AVS) of the National Parks Board.

“*The IACUC*”, which describes in detail the operational aspects pertaining to the Institutional Animal Care and Use Committee (IACUC). All institutions with research facilities are required to establish their own IACUC to assume this function. The IACUC is responsible for the oversight and evaluation of an institution’s animal care and use programme, and for ensuring that the care and use of animals for scientific purposes and all animal experimental procedures are in compliance with the *Guidelines*.

“*Training*”, which outlines the training scope and requirements for users of animals for research and animal facilities personnel. This section is to assist IACUCs in determining the scope and depth of the educational and training programmes that will meet both institutional needs and the requirements of NACLAR. This includes the scope of the core curriculum and the relevant core competencies, such as special courses for animal procedures. All users of animals for research should undergo appropriate training before carrying out any experiments using animals.

“*Occupational Health and Safety*”, which includes general information on occupational health and safety in animal care and use programmes, as well as more specific information on hazard identification and risk assessment; control and prevention strategies; managing animal experimentation involving hazards; medical evaluation and preventative medicine for personnel; and facilities, equipment and monitoring. Each institution should establish and maintain an occupational health and safety programme (OHSP) as an essential part of its animal care and use programme, which must be consistent with applicable legislation and guidelines.

This second edition of the *Guidelines* follows an extensive review of the first edition by NACLAR and its subcommittees. In addition, changes have been made as a result of feedback sought from stakeholders such as licensed research facilities. Some key changes include the expansion of the Guiding Principles to include a clearer definition of the Institutional Official (IO), changes in the programme review frequency, and the addition of a Designated Member Review (DMR) procedure. The IACUC section has been made more consistent, and where possible the regulatory burden has been reduced. Efforts have also been made to improve the welfare of the laboratory animals. The definition of staff categories needing training has been revised, and the training content has been reviewed to better fit the needs of animal facilities personnel.

The Appendices provide additional information covering the care and use of fish, amphibians and reptiles; the care and use of non-human primates; standards for housing and environmental conditions; and the signs of animal pain and distress.

The preparation of this second edition of the *Guidelines* has proven to be a monumental task, and I gratefully acknowledge the commitment and dedication of all members of NACLAR and the NACLAR Secretariat in this undertaking, as well as to the Chairpersons and members of the subcommittees overseeing the four sections. NACLAR also expresses its fullest appreciation to our colleagues in the AVS who are responsible for the implementation of the *Guidelines*, and to all our stakeholders who provided invaluable feedback to us. We would like to express our deep appreciation to Dr. Patricia V. Turner for serving as our Advisor and always readily responding to our queries. NACLAR is especially grateful to Dr. Michele Bailey who expertly and efficiently undertook the arduous task of editing this second edition of the *Guidelines*.

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NATIONAL ADVISORY COMMITTEE FOR LABORATORY ANIMAL RESEARCH GUIDELINES FOR THE CARE AND USE OF ANIMALS FOR SCIENTIFIC PURPOSES

SECTION I: INTRODUCTION

I.1 Background

- I.1.1 The National Advisory Committee for Laboratory Animal Research (NACLAR) was set up in 2003 to recommend guidelines for the care and use of animals for scientific purposes in Singapore. Members were appointed by the National Research Foundation (NRF). The NACLAR was re-convened in 2016 to review the guidelines. Members included representatives from academia, research, the National Parks Board/ Animal and Veterinary Service (NParks/AVS), veterinarians, the community, as well as legal and ethical specialists. A new section on the Occupational Health and Safety in Animal Care and Use was added. The revised guidelines consist of the following five sections:

Section I: Introduction

Section II: Guiding Principles

Section III: The Institutional Animal Care and Use Committee

Section IV: Training

Section V: Occupational Health and Safety in Animal Care and Use

The above sections must be taken together to form the NACLAR Guidelines.

- I.1.2 The NACLAR Guidelines were drawn up after review of the principles and practices adopted in other countries such as [Australia](#), [Canada](#), [New Zealand](#) and the [United States of America](#). See [Appendix I: Reference Materials](#) for these and other useful references.

I.2 Definitions of Terms

AAALAC International: A private, non-profit organization that promotes the humane treatment of animals in science through voluntary accreditation and assessment programs.

Adverse event: Any unexpected event that has a negative impact on the well-being of an animal used for scientific purposes.

Anaesthesia: A state of controllable, reversible insensibility in which sensor perception and motor responses are both markedly depressed.

Analgesia: The temporary abolition or diminution of pain perception.

Animal: All live fish, amphibians, reptiles, birds, non-human mammals and cephalopods.

Approved Protocol: Any project that involves the use of any animal for any scientific purpose and that is approved by an Institutional Animal Care and Use Committee (IACUC).

Aquatic Animals: Cephalopods, fish, aquatic or semiaquatic amphibians, reptiles and marine mammals.

Attending Veterinarian (AV): A veterinarian employed or contracted by an institution, on a full-time or part-time basis, with relevant training or experience in laboratory animal science and medicine to provide adequate veterinary care to the animals in the research facility of the licensee.

Cachexia: Poor body condition, emaciation, or severe physical wasting.

Death as an end-point: When the death of an animal is the deliberate measure used for evaluating biological or chemical processes, responses or effects.

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

Embryo: The early or developing stage of any organism, especially the developing product of fertilization of an egg.

Endangered species: Species included in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (see [CITES](#) in [Appendix I: Section II: General](#)).

Euthanasia: The act of inducing humane death in an animal.

Experimental endpoint: The point when scientific aims and objectives of the study have been reached, and the study is ended.

Holding facilities: Central or core buildings holding animals for more than 24 hours.

Housing facilities: Buildings, yards, paddocks or grounds in which animals are kept.

Humane endpoint: Criteria used to determine the point at which pain or distress in an experimental animal is prevented, terminated, or relieved prior to the planned experimental endpoint.

Institutional Animal Care and Use Committee (IACUC): The Committee, constituted by an institution, responsible for the oversight and evaluation of all aspects of the institution's animal care and use programme.

Institution: Any institution, company, organisation, association, body or person that uses or intends to use animals for scientific purposes and is licensed to do so.

Institutional Official (IO): The person who is in the position to obtain resources for the institution's animal care and use programme and to enforce the recommendations of the IACUC.

Licensee: A person who holds a valid licence issued by the Director-General, Animal Health and Welfare (who is currently also Director-General, NParks/AVS).

Manipulation: Any alteration of the normal physiological, behavioural or anatomical integrity of the animal depriving it of its usual care or subjecting it to a procedure which is unusual or abnormal; when compared with that to which animals of that type would be subjected to under normal management or practice. Should not include routine veterinary care.

Pain: An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.¹

Principal Investigator (PI): A person who proposes and/or has IACUC approval to conduct a protocol involving the use of animals, and has overall responsibility of a protocol.

Perioperative: The period around the time of a surgical procedure including the preoperative, intraoperative and postoperative phases.

Procedure areas: Locations where animals undergo any procedure and/or are held for less than 24 hours.

Programme: Refers to relevant policies, procedures, standards, organisational structure, staffing, facilities and practices established in the institution pertaining to the care and use of animals for scientific purposes.

Protocol: A written description of a project put forward by a PI for review by an IACUC.

¹ IASP Task Force on Taxonomy, 1994. Part III: Pain Terms, A Current List with Definitions and Notes on Usage. In H. Merskey, & N. Bogduk (Eds.), *Classification of Chronic Pain*. IASP Press.

This term has been reproduced with permission of the International Association for the Study of Pain ® (IASP). The term may not be reproduced for any other purpose without permission.

Research facility: All the premises of the licensee which are approved under the licence for the keeping or use of animals for scientific purposes, including all animal housing, holding and satellite facilities, and all animal procedure areas.

Satellite facilities: Buildings, other than central or core holding facility, which hold animals for more than 24 hours.

Scientific Purposes: Any purpose for which activities (including breeding of animals integral to a research or teaching project) are performed for the acquisition, development or demonstration of knowledge or techniques in any scientific discipline, including the purpose of research, teaching, diagnosis, field trials, product testing, production of biological products and environmental studies.

Sedation: A drug-induced state characterised by decreased awareness of surroundings, relaxation, and sleepiness.

Staff: Persons including PIs, animal facility and research staff, and students involved in the housing, feeding, care and use of animals.

Teaching activity: Any action or group of actions undertaken with the aim of imparting or demonstrating knowledge or techniques to achieve an educational outcome, as specified in the relevant curriculum or competency requirements.

Tranquillisers: Drugs used to reduce anxiety or produce sedation.

Wildlife: All species of animals from free populations whether indigenous or otherwise.

I.3 Definitions of ‘May’, ‘Should’ and ‘Must’, as used in the NACLAR Guidelines

I.3.1 **May** indicates a discretionary action.

I.3.2 **Should** indicates a strong recommendation for achieving a goal. However, there may be valid reasons in particular circumstances to justify an alternative strategy, but the full implications must be understood and weighed before choosing the alternative.

I.3.3 **Must** indicates actions that are an absolute requirement.

I.4 Acronyms

AALAS	American Association for Laboratory Animal Science
ABSL-2	Animal Biosafety Level 2
ABSL-3	Animal Biosafety Level 3
ABSL-4	Animal Biosafety Level 4
ACLAM	American College of Laboratory Animal Medicine
ALAT	Assistant Laboratory Animal Technician
AV	Attending Veterinarian
AVS	Animal and Veterinary Service
BATA	Biological Agents and Toxins Act
BSO	Biosafety Officer / Coordinator
CCAC	Canadian Council on Animal Care
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CMAR	Certified Manager of Animal Resources
CPIA	Certified Professional in IACUC Administration
DMR	Designated Member Review
FCA	Freund’s Complete Adjuvant
GM	Genetically Modified
GMAC	Genetic Modification Advisory Committee
GMO	Genetically Modified Organism
HBRA	Human Biomedical Research Act
IACUC	Institutional Animal Care and Use Committee
IATA	International Air Transport Association
IBC	Institutional Biosafety Committee
IFA	Incomplete Freund’s Adjuvant
ILAM	Institute for Laboratory Animal Management
IO	Institutional Official
IRB	Institutional Review Board
LAT	Laboratory Animal Technician
LATG	Laboratory Animal Technologist
MSS	Multiple Survival Surgeries
NACLAR	National Advisory Committee for Laboratory Animal Research
NC3Rs	National Centre for the Replacement & Reduction of Animals in Research
NHP	Non-Human Primate

NIH	National Institutes of Health (USA)
NIOSH	National Institute of Occupational Safety and Health (USA)
NParks	National Parks Board
NRF	National Research Foundation
OACU	Office of Animal Care and Use (NIH Intramural Research Program)
OHSP	Occupational Health and Safety Programme
OLAW	Office of Laboratory Animal Welfare (NIH)
PAM	Post Approval Monitoring
PI	Principal Investigator
PPE	Personal Protective Equipment
PRIM&R	Public Responsibility in Medicine and Research
RCULA	Responsible Care and Use of Laboratory Animals
SFA	Singapore Food Agency
SOP	Standard Operating Procedure
VAS	Visual Analogue Scale
WSHA	Workplace Safety and Health Act

SECTION II: GUIDING PRINCIPLES

II.1 Purpose of the Guiding Principles

II.1.1 The purpose of the Guiding Principles is to:

- a. set out the framework for the NACLAR Guidelines, and
- b. promote the humane and responsible care and use of animals for scientific purposes in accordance with the principles of replacement, reduction and refinement.

II.2 Scope of the NACLAR Guidelines

II.2.1 The NACLAR Guidelines cover all live vertebrates; including fish, amphibians, reptiles, birds and non-human mammals, as well as cephalopods.

II.2.2 As a general guide, when the embryos and fetuses of the animals listed in [II.2.1](#) are at the developmental stage where they begin to feel pain, they become covered by the NACLAR Guidelines. NACLAR does not prescribe this exact day or stage of development. The Principal Investigator (PI) and the Institutional Animal Care and Use Committee (IACUC) are responsible for developing policies in line with current best practices and scientific literature.

II.2.3 The NACLAR Guidelines cover all aspects of the care and use of animals for scientific purposes including their use in teaching, field work, environmental studies, research, diagnostics, product testing, and the production of biological products.

II.2.4 The NACLAR Guidelines outline the responsibilities of institutions, IOs, PIs, staff and others involved in the care and use of animals for scientific purposes.

II.3 Framework to Comply with the NACLAR Guidelines

II.3.1 The institution, Institutional Official (IO), PIs, staff and other persons involved in the care and use of animals for scientific purposes must comply with all current and relevant guidelines and regulations.

II.3.2 The NACLAR Guidelines are based on the premise of self-regulation by the institution. Thus, the institution must take responsibility for complying with the NACLAR Guidelines. Each institution must establish an IACUC, which in turn must ensure and verify that the use and care of animals for scientific purposes are in accordance with the NACLAR Guidelines.

II.3.3 PIs are to ensure their protocols are in accordance with the NACLAR Guidelines and the approval given by the IACUC.

II.3.4 Keeping of records and reports

- a. All records, reports and documentation relating to the keeping and use of animals for scientific purposes must be maintained in accordance with the NACLAR Guidelines and kept for a minimum of 3 years after the termination of the project, unless otherwise required.
- b. All such records, reports and documentation must at reasonable times be made available for inspection and copying by the Director-General, Animal Health and Welfare (who is currently also Director-General, NParks/AVS).
- c. Licensees who contravene the above shall be guilty of an offence and shall be liable to be punished in accordance with the Rules (see [Rule 13 and Rule 19](#) in [Animals and Birds \(Care and Use of Animals for Scientific Purposes\) Rules](#) in [Appendix I: Reference Materials: Legislations](#)).

II.4 General Principles for the Care and Use of Animals for Scientific Purposes

II.4.1 General Principles

- II.4.1.1 Protocols involving animals must be designed and undertaken only after due consideration of their value to human and/or animal health, or the advancement of knowledge, and weighed against the potential effects on the welfare of the animals.
- II.4.1.2 PIs and staff must treat animals as sentient and must regard their proper care and use and the avoidance or minimisation of discomfort, distress, or pain as imperatives.
- II.4.1.3 The approach known as the 3Rs must be considered at all times:
 - a. *Replacement* of animals with non-animal methods, or animals of a lower phylogeny.
 - b. *Reduction* in the number of animals used.
 - c. *Refinement* of techniques used to minimise impact on animals and promote positive well-being.

II.4.2 Replacement of animal experimentation with alternative methods

- II.4.2.1 Alternative methods, such as mathematical models, computer simulation and *in vitro* biological systems, which may replace or complement the use of animals must be considered before embarking on any protocol involving the use of animals.

II.4.3 Reduction in the number of animals used

- II.4.3.1 The number of animals used must be the minimum and appropriate number required to obtain scientifically valid results.
- II.4.3.2 The principle of reducing the number of animals should not be implemented at the expense of greater suffering of individual animals.
- II.4.3.3 Scientific activities involving the use of animals must not be duplicated, repeated or replicated unnecessarily.
- II.4.3.4 Breeding of animals must be managed to avoid, or minimise, the production of excess animals.
- II.4.3.5 Reduction of animal numbers may be accomplished by a variety of methods as described in the table below:

Method	Examples
Rational selection of group size	Pilot studies to estimate variability and evaluate procedures and effects.
Careful experimental design	<p>Appropriate choice of control groups.</p> <p>Standardising procedures to minimise variability.</p> <p>Maximising the use of each animal, as appropriate.</p> <p>Performing several terminal procedures per animal while under anaesthesia.</p> <p>Animals euthanised by one PI providing tissue samples needed by another PI.</p>
Correct choice of model	Use of healthy, genetically similar animals to decrease variability.
Minimising loss of animals	Good peri-operative care.
Controlled breeding of animals	<p>Avoid unintended breeding.</p> <p>Plan ahead so the appropriate number of animals needed for studies are ordered or bred.</p>
Statistical analysis	Appropriate use of statistical software to generate the maximum information from the minimum, but sufficient, number of animals to obtain valid results.

II.4.4 Refinement of protocols and techniques used to minimise impact on animals

- II.4.4.1 Animals must be of an appropriate species and quality for the protocol, considering their biological characteristics, including behaviour, genetic constitution; and nutritional, microbiological and general health status.
- II.4.4.2 Protocols must be designed to avoid or minimise pain or distress to animals.
- II.4.4.3 Unless contrary to scientific evidence, it must be assumed that procedures that will cause pain or distress in human beings will cause pain or distress in animals.
- II.4.4.4 If there is no existing scientific evidence or consensus as to whether a certain procedure actually causes pain or distress in the affected animals, the IACUC may need to utilise one or more of the following to facilitate their deliberations:
 - a. pilot studies,
 - b. evaluations of clinical signs,
 - c. clinical pathology,
 - d. gross and histological necropsy studies,
 - e. review of comparable literature, and/or
 - f. consultation with experts.
- II.4.4.5 If there remains any doubt about the potential for, or actual presence of, pain or distress, the IACUC should err on the side of protecting the animals against the possibility of unnecessary pain or distress.
- II.4.4.6 Procedures causing more than momentary or slight pain or distress, when performed on animals, should be performed with appropriate sedation, analgesia, and/or anaesthesia in accordance with accepted veterinary practice.
- II.4.4.7 Surgical or other painful procedures must not be performed on unanaesthetised animals that have been paralysed by chemical agents, unless these unanaesthetised animals have undergone an appropriate surgical procedure that eliminates sensory awareness.
 - a. If chemical agents are used to paralyse the animal, continuous or frequent intermittent monitoring of the paralysed animal is essential to ensure that analgesia is adequate to prevent pain or distress.

- II.4.4.8 At the end of, or, when appropriate, during the procedures, animals that would otherwise suffer severe or chronic pain or distress, that cannot be relieved promptly, must be euthanised.
- II.4.4.9 An animal that develops signs of pain or distress of a kind and degree not predicted in the approved protocol, must have the pain or distress alleviated promptly. If severe pain or distress cannot be alleviated promptly, the animal must be euthanised.
- II.4.4.10 If it is not possible to use anaesthetics or analgesics in any protocol (or part of a protocol), the end-point of the protocol must be as early as possible to avoid or minimise pain or distress to the animals.
- II.4.4.11 Protocols involving the use of animals must be as brief as possible whilst being consistent with achieving the scientific goal(s).
- II.4.4.12 The transportation, housing, feeding and handling of animals should meet species-specific needs, including behavioural and biological needs.
- II.4.4.13 The result of previously published information or pilot studies, if there is any, as well as information about pharmacology and chemistry of the compound being tested, must be used to predict any potential adverse effects on animals.

II.5 Animal Housing and Management

References are provided in [Appendix I: Section II: Guiding Principles](#).

II.5.1 General

- II.5.1.1 Housing facilities where animals are kept must be appropriately staffed, designed, constructed, equipped and maintained to achieve a high standard of animal care, fulfil scientific requirements and comply with the NACLAR Guidelines.
- II.5.1.2 In general, housing and management practices should be designed to provide a high standard of animal care based on the criterion of animal well-being for the respective species used.
- II.5.1.3 The standard of animal care must be maintained at all times, including weekends and holidays.
- II.5.1.4 Emergency care must be available at all times.
- II.5.1.5 As the security of animals directly impacts their welfare, housing facilities must be secured with controlled access.

- II.5.1.6 Outdoor housing facilities must be compatible with the needs of the species, provide adequate shelter and water, protect the animals from predation, and comply with farm, zoological garden or general outdoor housing best practices.
- II.5.1.7 Indoor housing facilities must be compatible with the needs of the species to be housed.
- II.5.1.8 Housing facilities should be designed and operated to control environmental factors, exclude vermin, and limit contamination.
- II.5.1.9 Housing facilities must be maintained in good condition.
- II.5.1.10 Walls and floors should be constructed of durable materials with surfaces that can be cleaned and disinfected readily.
- II.5.1.11 Housing facilities must be kept clean and tidy, and operated to achieve appropriate hygiene.
- II.5.1.12 There must be a pest control programme to monitor and control vermin.
- II.5.1.13 There must be adequate and appropriate storage areas for food, bedding, consumables and equipment.
- II.5.1.14 The choice of detergents, disinfectants and pesticides should be appropriate for the specific purpose and animal species housed.
- II.5.1.15 Agents designed to mask animal odours must not be used as they may expose animals to volatile compounds, which can alter metabolic processes. These agents are also not a substitute for good cage and equipment cleaning practices and appropriate ventilation.
- II.5.1.16 Cleaning practices should be monitored on a regular basis to ensure effective hygiene and sanitation. This should include visual inspection, monitoring water temperatures and microbiological testing of surfaces after cleaning.
- II.5.1.17 Appropriate water supply and drainage must be provided.
- II.5.1.18 Contingency plans to cover emergencies such as flooding and fire, or the breakdown of lighting, heating, cooling or ventilation must be in place.

II.5.2 Environmental factors

- II.5.2.1 Environmental factors affect the welfare of animals and may affect the results of experiments. Animals must be provided with environmental conditions appropriate to their behavioural and biological needs, unless contrary conditions are approved by the IACUC for the purposes of a protocol.

- a. Additional information on the care and use of aquatic animals for scientific purposes can be found in [Appendix II: Additional Information on the Care and Use of Fish, Amphibians and Reptiles for Scientific Purposes](#).

II.5.2.2 Air changes, temperature, humidity, noise, light intensity and light cycles must be maintained within limits compatible with the health and well-being of the animals. Refer to [Appendix III: Standards for Housing and Environmental Conditions](#).

II.5.2.3 Effective ventilation is essential for the welfare of animals and the control of temperature, humidity, odours and allergens.

- a. Ventilation systems should distribute air uniformly and achieve adequate air exchange, both within cages and within a room.
- b. Air pressurisation (directional air flow) assists in controlling airborne contamination and odours. The air pressurisation must be appropriate for the animal species and health status, the use of the room, as well as protocol and safety needs.
- c. Environmental fluctuations should be minimised.
 - i. Exposure to temperature and humidity fluctuations results in behavioural, physiological, and morphological changes, which negatively affect animal well-being and research outcomes.

II.5.2.4 Noxious and potentially harmful waste gases, particularly ammonia, should be kept to a level compatible with the health and comfort of the animals. The adequacy of the ventilation system, placement of cages, population density, number of cages, effectiveness of housekeeping and frequency of bedding changes will all influence the level of noxious gases.

II.5.2.5 The IACUC, PIs and IOs should be informed in advance of planned changes to the environmental conditions of the housing facilities.

II.5.3 Pens, cages, other primary enclosures and the immediate environment of the animals

II.5.3.1 Pens, cages and other primary enclosures should be designed, constructed and maintained to ensure the comfort and well-being of the animals, considering the following factors:

- a. species-specific behavioural requirements, including free movement and activity, sleep requirements, privacy, and contact with others of the same species;

- b. species-specific environmental requirements such as lighting, temperature, air quality, appropriate day/night cycles and protection from excessive noise and vibrations;
- c. provision of social housing, which is considered the standard method of housing for social species;
 - i. Single housing of animals should only occur when it is appropriate for the species, due to social incompatibility, or veterinary concerns regarding animal well-being or if there is adequate scientific justification for the purpose of the experiment that has been approved by the IACUC.
 - ii. Where it is necessary to individually house social animals, the conditions should be managed so as to minimise the impact of social isolation.
 - a) Where possible, visual, auditory, olfactory and, depending on the species, protected tactile contact with compatible conspecifics should be provided.
 - b) In the absence of other animals, additional enrichment should be offered. This may include safe and positive interaction with the animal care staff, as appropriate to the species of concern; periodic release into larger enclosures; supplemental enrichment items; and/or the addition of a companion animal in the room or housing area.
 - iii. Isolation must be kept to a minimum period necessary.
 - iv. The institution's policy and exceptions from social housing should be reviewed on a regular basis and approved by the IACUC.
- d. the need to provide ready access to food and water;
- e. the need to clean the pen, cage or other primary enclosure;
- f. protection from spread of pests and disease;
- g. requirements of the experiments; and
- h. the need to observe the animals readily.

II.5.3.2 Pens, cages and other primary enclosures must also:

- a. be constructed of durable, impervious materials;
- b. be kept clean;

- c. be maintained in good condition;
 - d. be escape-proof;
 - e. protect the animals from climatic extremes;
 - f. not cause injury to the animals; and
 - g. be large enough to ensure the animals' well-being – i.e. allow for expression of species-specific behaviours and postural adjustments.
 - h. Refer to [Appendix III: Standards for Housing and Environmental Conditions](#).
- II.5.3.3 Wire floor cages should not be used unless they are essential to the project and approved by the IACUC. If used, wire floor cages should only be used for brief periods. Animals should be provided with a solid floor area for resting when housed in wire floor cages.
- II.5.3.4 The population density of animals within cages, pens or other primary enclosures and the placement of these in rooms should be such that acceptable social and environmental conditions for the species can be maintained.
- II.5.3.5 Bedding and litter should be provided as appropriate to the species, and should be comfortable, absorbent, dust-free, non-palatable, non-toxic, as well as sterilisable (if needed).
- II.5.3.6 Pregnant animals must be provided with nesting materials when appropriate for the species.
- II.5.3.7 Changes in housing conditions may affect the welfare of the animals and the results of experiments. The IACUC and PIs should be informed in advance of planned changes to the housing conditions.
- II.5.3.8 The research facility should have standard operating procedures (SOPs) for the recapture of escaped animals that set out the reporting procedure. If an animal escapes from the facility, the IACUC must be informed immediately. The facility is expected to employ all measures necessary to recapture the animal. If the facility is unable to recapture the animal within 24 hours, NParks/AVS should be notified.

II.5.4 Enrichment and environmental complexity

II.5.4.1 Most animals used for scientific purposes are housed in environments dissimilar to their natural habitats. Wherever possible, such animals should be provided an enriched environment that promotes the expression of normal behaviour appropriate to the species. Environmental enrichment is aimed at enhancing animal well-being by improving the quality of the captive environment with provision of stimuli, structures and resources to allow animals to express a range of species-typical behaviours and/or give animals more control over their environment.

II.5.4.2 Behavioural management techniques have often been categorised into the following five broad and overlapping categories with variable applicability depending on species:

- a. **Social Housing:** Most species have well defined social structures and prefer to live in groups. Thus social housing should be the default for social species, although care must be taken to ensure that animals are socially compatible. Refer to [II.5.3.1.c](#).
- b. **Exercise:** Increasing the complexity of the physical environment with appropriate devices for the species may enhance animal well-being by providing opportunities for physical exercise.
- c. **Physical environment:** Provision of structures, such as lofts, perches, shelves, tunnels, visual barriers, shelters, or nest boxes provide places to flee or hide. Novel devices, such as nesting materials, toys, chew sticks, puzzles, or foraging device provide tactile stimulation and allow animals to express normal behaviours.

Enrichment methods or devices that provide visual, auditory, or olfactory stimulation may promote animal well-being. These may include TV/videos, music, nature sounds, or scents. As with most enrichment methods, upon introduction of sensory enrichment the animals should be monitored to detect any aversive, anxiety-like or antagonistic behavioural responses.

- d. **Food Enrichment:** In a non-captive environment most species spend a large amount of time foraging for a variety of foods. However, in a captive environment, animals generally receive a standard diet provided in a readily accessible feeder. Presenting the food in a way that requires more time and effort for the animal to obtain may be enriching for some species. Supplementing the animal diet with other palatable food items, such as fruits, vegetable, nuts, hay or commercially available enrichment treats may also be considered. Such treats may also be presented in a manner that promotes foraging behaviour or rewards positive behaviour. The amount of food enrichment should be limited to prevent dietary imbalances and/or excess caloric intake leading to malnutrition or obesity.

- e. **Cognitive/Occupational Enhancements:** Animals in the wild face challenges related to finding food, mates, shelter, and evading predators. Some species may benefit by being presented with challenges that require a toolkit of cognitive skills such as exploration, problem-solving, various forms of learning and spatial awareness. For example, using positive reinforcement to train animals to cooperate with husbandry and/or experimental procedures may promote well-being by giving the animal a sense of control over its environment.

II.5.4.3 Novelty of enrichment through rotation or replacement of items or activities should be a consideration. However, changing an animal's environment too frequently may be stressful and some items may cause a stress response in some species. A species' need for familiarity of scents, items or structures should be considered when considering rotation or replacement of enrichment items. Some species are fearful of certain novel items. The behavioural management program should be customised for each species and may need to be adjusted based on animal response.

II.5.4.4 Behavioural management/enrichment programs should be reviewed by the IACUC, researchers, and the attending veterinarian (AV) on a regular basis to ensure that they are beneficial to animal well-being and consistent with the goals of animal use.

II.5.5 Food and water

II.5.5.1 Animals should receive appropriate, uncontaminated and nutritionally balanced food according to accepted requirements for the species.

II.5.5.2 Food should be provided daily (or according to the animal's requirements) in sufficient quantity and of appropriate composition to maintain normal growth of immature animals, normal weight of adult animals or provide for the requirements of pregnancy or lactation.

II.5.5.3 When animals are fed in groups, there should be sufficient trough space or feeding points to cater to the number and size of animals that eat together at one time to avoid undesirable competition for food, especially if feed is restricted.

II.5.5.4 Uneaten perishable food should be removed unless contrary to the eating habits or needs of the species.

II.5.5.5 Any alteration to dietary regimes should be gradual when possible.

- II.5.5.6 Food should be stored so as to minimise deterioration of nutritional value and palatability and to prevent contamination by vermin. Storage of natural-ingredient diets at less than 21°C (70°F) and below 50% relative humidity is recommended.
- II.5.5.7 Drinking water should always be available, and be clean, fresh and uncontaminated. Water sources should be designed to prevent faecal contamination.
- II.5.5.8 Feed and water equipment should be constructed of materials that can be easily and effectively cleaned.
- II.5.5.9 Variations to the above requirements ([II.5.5.1-8](#)) must receive IACUC approval.

II.5.6 Identification of animals

- II.5.6.1 Animals should be identified by a method such as tattoo, neck-band, individual tag, electronic numbering device, physical mark or by a label or marking attached to the cage, container, pen, yard or enclosure in which the animals are kept.
- II.5.6.2 The method of identification used should be reliable and chosen to minimise stress, or subsequent injury, to the animal.

II.5.7 Disposal of animal carcasses and waste

- II.5.7.1 Appropriate provision must be made for prompt and sanitary disposal of animal carcasses and waste material in accordance with current laws and any other guidelines or requirements of the National Biosafety Committee, the Ministry of Health and the National Environmental Agency (see [MOH: Biosafety Committee](#), and [NEA](#) in [Appendix I: Section II: General](#)).

II.5.8 Quarantine and isolation facilities

- II.5.8.1 Institutions should have quarantine and isolation facilities to allow for the effective segregation of animals. These facilities could be used for the admission of new animals for disease control.
 - a. The need for quarantine must be determined by the AV and is based on the species, source, health status, and other relevant criteria.
- II.5.8.2 New animals should be immediately inspected by the AV or his/her designee, and then placed in quarantine, if appropriate. The duration and SOPs for quarantine at the facility should be risk-based, and determined in consultation with the AV. The new animals should be evaluated in terms of:

- a. health. To assess the health status of rodents and for some other species, the AV may obtain a certificate from the supplier stating which pathogens had been tested within the last 6 months, or as determined by the AV, and the results of the tests. Animals imported from approved sources overseas may come with a health certificate endorsed by the exporting country's competent authority.
- b. suitable condition or health status for proposed projects.

II.5.8.3 Animals in quarantine should generally not be used for any project unless there is strong justification approved by the IACUC.

II.5.9 Housing standards

II.5.9.1 Apart from the general principles set out above, there are also detailed standards for housing, environmental conditions and other physical facilities set out in [Appendix III: Standards for Housing and Environmental Conditions](#); which PIs, AVs and staff should be familiar with and apply.

II.5.9.2 Where standards have not been set out, judicious extrapolation from existing knowledge and consultation with veterinarians, laboratory animal specialists and other relevant individuals should be done to arrive at housing and environmental setups that will be conducive to the well-being of the animals.

II.5.10 Non-human primates

II.5.10.1 Non-human primates (NHPs) are recognised as having highly-developed mental and emotional capacities. In many ways, they are similar to humans and exhibit many similar external manifestations of emotions such as vocalisations, postures, gestures, and reactions. PIs, AVs and staff should familiarise themselves with the references and information contained in Appendices [I: Section II: Non-Human Primates](#) and [IV: Additional Information on the Care and Use of NHPs for Scientific Purposes](#).

II.5.11 Provisions for animals at the conclusion of their use on a protocol

II.5.11.1 Provisions for animals at the end of their use on a protocol may include:

- a. **Rehousing (rehoming):** Opportunities to rehome animals should be considered whenever 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. An animal must not be released to a person, institution or organisation, at the conclusion of their use unless:

- i. the IACUC has approved such release;
 - ii. safeguards are in place and approved by the IACUC;
 - a) In the case of persons under the age 21, safeguards must include a written commitment from a parent or guardian for the provision of adequate, ongoing and responsible care of the animal.
 - iii. all persons involved in the rehoming process must demonstrate an awareness of relevant legislative requirements regarding the animal being rehomed, in particular, the Animals and Birds (Dog Licensing and Control) Rules (see [Animals and Birds \(Dog Licensing and Control\) Rules](#) in [Appendix I: Reference Materials: Legislations](#));
 - iv. transport of animals between sites is in accordance with the NACLAR Guidelines (refer to [II.6.4](#)); and
 - v. all animals rehomed are on NParks/AVS's permitted list of animals (refer to NParks/AVS website).
- b. **Euthanasia:** must be in accordance with the NACLAR Guidelines on humane and experimental endpoints (refer to [II.11.5](#)) and AVMA *Guidelines for the Euthanasia of Animals* (see [AVMA Panel on Euthanasia](#) in [Appendix I: Section II: Euthanasia](#)). Refer to [II.11.9](#) and [III.4.3](#).
 - c. **Reuse:** in accordance with the NACLAR Guidelines. Refer to [II.11.6](#).
 - d. **Tissue sharing:** where practicable and for scientific reasons, for example, PIs may share, with other PIs, tissues from euthanised animals.

II.6 Procurement and Transport of Animals

II.6.1 Source and admission of new animals

- II.6.1.1 All laboratory animals obtained locally must be from a licensed animal research facility or otherwise legally permitted source.
 - a. For animals such as dogs and cats, and farm animals such as pigs, sheep, goats and cattle, the animals should be properly identified (e.g. microchip number) and the supplier must have appropriate papers to prove legal ownership of the animals.
- II.6.1.2 All laboratory animals obtained from overseas must be from sources approved by NParks/AVS. No animal is to be imported without a licence from NParks/AVS as required under the *Animals and Birds Act (Cap.7)* (see [Animals and Birds Act](#) in [Appendix I: Reference Materials: Legislations](#)).

- a. NParks/AVS may impose quarantine requirements for specific species of imported laboratory animals or consignments at the time of import. The duration and location of the quarantine will be determined by NParks/AVS.

II.6.1.3 For more information on admission of animals into the facility, refer to [II.5.8](#).

II.6.1.4 The transport, import and use of all genetically modified (GM) animals must be in accordance with the Singapore Biosafety Guidelines for Research on Genetically Modified Organisms (GMOs), as set out by the Genetic Modification Advisory Committee (GMAC) (see [GMAC](#) in [Appendix I: Section II: General](#)). Refer to [III.4.8](#).

II.6.2 Considerations in procurement of CITES-listed animals

II.6.2.1 No CITES-listed animal is to be imported without proper CITES certificates, export permits from the exporting country and import permits from NParks/AVS, as required under the *Endangered Species (Import and Export) Act (Cap.92A)* (see [Endangered Species Act](#) in [Appendix I: Reference Materials: Legislations](#)).

II.6.2.2 An endangered animal that is included in CITES (see [CITES](#) in [Appendix I: Section II: General](#)), must not be used in protocols unless the protocols concerned will be of direct benefit to the conservation of that species or a closely related species, and will not further endanger the species.

II.6.3 Considerations in procurement and use of wildlife

II.6.3.1 Animals may be taken from natural habitats only if animals bred in captivity are not available or are unsuitable for the specific scientific activity.

II.6.3.2 Under [Wildlife Act \(Cap. 351\)](#) (see [Appendix I: Reference Materials: Legislations](#)), all wildlife is protected by law unless otherwise stated. An approval must be obtained from the Director-General (Wildlife Management) to kill, trap, take, keep, feed, release, import, export, or offer for sale wildlife.

II.6.3.3 Capture and restraint are stressful to animals in the wild. Skilled persons must be used, and strategies must be employed to minimise distress during capture and disruption of the colonies from which they are taken.

- a. There must be careful choice of suitable capture techniques, and appropriate and safe enclosures or caging must be used.
 - i. Animals must be monitored for signs of distress following capture and appropriate measures taken to minimise the stress.

- ii. Any animal suffering from capture-induced trauma should receive treatment without delay.

II.6.3.4 When devices are used to track the movement of wildlife, the weight, design and positioning of attached devices must minimise interference with the normal survival requirements of the animal.

II.6.3.5 The considerations stated in section [II.6.3](#) apply to the use of stray and feral animals in field studies and other scientific activities.

II.6.4 Transport of animals

II.6.4.1 Transportation can cause distress due to confinement, movement, noise, changes in the environment and contact with personnel.

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

II.6.4.2 The animal must be provided with adequate shelter, food and water and must have sufficient space to lie down, stand and stretch.

II.6.4.3 During local transport, animals must be transported under conditions that are appropriate to the species and that meet standards generally adopted in veterinary and laboratory animal medicine so as to ensure that the welfare of the animals is not unduly compromised.

II.6.4.4 Potential sources of distress should be identified and steps taken to avoid or minimise their effects on the animals.

II.6.4.5 For aquatic species and amphibians, special considerations are required for transportation in an aqueous or sufficiently moist environment, and special attention should be given to avoiding temperature extremes for poikilotherms.

II.6.4.6 Containers must be escape and tamper-proof and there should be adequate nesting or bedding material where appropriate.

II.6.4.7 Animals should be protected from sudden movements and extremes of climate.

II.6.4.8 Both the suppliers and recipients of animals must ensure that delivery procedures are satisfactory.

- a. Suppliers should notify recipients of transport details and estimated time of arrival to ensure swift delivery.

- b. Research facilities must ensure that animals are received by a responsible person and transferred to appropriate accommodation without delay.

II.6.4.9 The transfer of GM animals between approved institutional containment facilities must be in accordance with the guidelines set out by GMAC (see [GMAC](#) in [Appendix I: Section II: General](#)).

II.6.4.10 Transport by air must be in accordance with International Air Transport Association (IATA) regulations, or other applicable regulations (see [IATA](#) and other relevant regulations in [Appendix I: Section II: General](#)).

II.6.4.11 Mode of transport of animals must address animal biosecurity, safety, health, and liability risks for the animals, personnel, and the institution.

II.7 Staff at Research Facilities

II.7.1 Staff

II.7.1.1 A very important factor for ensuring high standards of animal care is to have a sufficient number of well-trained, knowledgeable and committed staff.

II.7.1.2 Staff working with animals should be appropriately instructed in the care and maintenance of those animals. They should appreciate their role in facilitating the well-being of the animals and the successful outcome of projects.

II.7.1.3 Staff should be instructed on how to recognise at an early stage, changes in animal behaviour, performance and appearance.

II.7.1.4 Institutions must encourage and promote formal training of all staff in animal science and/or technology.

II.7.2 Supervisor

II.7.2.1 The supervisor of animal care staff must have the appropriate veterinary or animal care qualifications, and/or experience in handling of the species concerned.

II.7.3 Training for Staff

II.7.3.1 The minimum training requirements for staff and other recommended training are set out in [IV.2](#).

II.8 Veterinary Care

II.8.1 Attending Veterinarian (AV)

- II.8.1.1 Each Institution must have an AV to advise on the appropriate care and use of animals and provide adequate veterinary care.
- II.8.1.2 Each institution must give the AV authority to provide appropriate veterinary care.
- II.8.1.3 In the case of a pressing health problem, if the responsible person (e.g. researcher or PI) is not available or if the responsible person and veterinary staff cannot reach consensus on treatment, the AV must have the delegated authority by the institution, and the IACUC, to treat an animal, remove it from an experiment, institute appropriate measures to relieve severe pain or distress or perform euthanasia if necessary.
- II.8.1.4 There must be formal communication channels between the AV, IO and the IACUC Chair.
- II.8.1.5 The AV must be employed under formal arrangements. The AV may be employed on a part-time or full-time basis.
- II.8.1.6 The institution must arrange with the AV to provide a written programme of veterinary care requirements to be complied with. In the case of a part-time AV, the written programme must also set out regularly scheduled visits to the housing and animal procedure areas.
- II.8.1.7 If the AV is on leave or will be otherwise unavailable to provide any general or emergency veterinary care, interim arrangements must be made to ensure that there is always ready access to veterinary care.
- II.8.1.8 The AV and all veterinarians with delegated AV responsibilities, employed on a full-time, part-time or contract basis, must be qualified in veterinary science and be licensed by NParks/AVS.
- II.8.1.9 The AV or his/her designee, should investigate unexpected or unintended morbidity and mortality to determine the cause and initiate remedial action, if appropriate.

II.8.2 Components of veterinary care

- II.8.2.1 There must be ready access to veterinary care for all animals at all times. Institutions must establish and maintain adequate veterinary care (refer to [ACLAM](#) in [Appendix I: Guiding Principles: Veterinary Care](#)), overseen by the AV, including:

- a. the availability of appropriate facilities, personnel, equipment, and services to comply with the NACLAR Guidelines.
- b. the use of appropriate methods to prevent and control diseases (e.g. vaccination and other prophylaxis, disease monitoring and surveillance, quarantine and isolation), diagnose diseases, and treat various conditions, diseases and injuries. Such methods include having robust biosecurity procedures appropriate for the protocols and animals used.
- c. the availability of 24-hour emergency care (including weekends and public holidays).
- d. daily observation (or more frequently, as necessary) of all animals to assess their health and well-being: The daily observation of animals may be accomplished by someone other than the AV provided that there is a mechanism of direct and frequent communication between the AV and the staff concerned so that timely and accurate information on problems of animal health, behaviour, and well-being is conveyed to the AV.
- e. guidance to PIs and other personnel involved in the care and use of animals regarding handling, immobilisation, anaesthesia, analgesia, tranquillisation, and euthanasia.
- f. adequate peri-operative (pre-procedural, surgical, and post-procedural) care in accordance with current established veterinary medical and nursing procedures.

II.8.3 Training for Veterinarians

- II.8.3.1 The minimum training requirements for veterinarians and other training that is recommended are set out in [IV.2.5](#).

II.9 Responsibilities of the Institutional Official (IO)

II.9.1 Overview

- II.9.1.1 The IO is to ensure the institution's compliance with the NACLAR Guidelines.
- II.9.1.2 Where the IO is a licensee, the IO must establish an IACUC and appoint the IACUC members. Otherwise, this should be done by the licensee.
- II.9.1.3 IACUCs are to report to the IO.

II.9.2 Responsibilities of the IO – Refer also to [III.1.3](#)

II.9.2.1 Through the IO, each institution must:

- a. Ensure, through the IACUC, that the care and use of animals for scientific purposes complies with the NACLAR Guidelines and relevant legislations.
- b. Provide the IACUC with facilities, authority, and resources to fulfil its terms of reference and responsibilities. Resources include the provision of educational materials, access to training courses for IACUC members and access to administrative assistance.
- c. Refer to the IACUC for comment on all matters that may affect animal welfare including the building and modification of housing and research facilities.
- d. Review annually the operation of the IACUC. This review includes an assessment of the annual report from the IACUC and a meeting with the IACUC Chair. This annual review of the IACUC is to help ensure that the IACUC is adjusting its operations in light of their experiences and circumstances, and according to continuing developments in the care and use of animals for scientific purposes.
- e. Respond effectively to recommendations from the IACUC to ensure that the facilities for housing, care and use of animals are appropriate for the maintenance of the health and well-being of the animals and that the disposal of the animals are appropriate.
- f. Respond promptly and effectively to recommendations from the IACUC.
- g. Establish appeal procedures for IACUC members and PIs who are dissatisfied with IACUC procedures or decisions.
- h. Put provisions in place to protect the identity of informers who report illegal and/or unethical practices.
- i. Ensure that the IACUC develops guidelines for animal care and use within the institution and that these are implemented, including ensuring that disease outbreaks and emergencies are detected promptly and dealt with effectively.
- j. Ensure that appropriate veterinary care is available for the animals and that there is access to diagnostic services.

II.9.3 Annual report by the institution

II.9.3.1 The Institution must provide an annual report to NParks/AVS, covering the period 1 January to 31 December of each year, failing which sanction could result under rule 14 read with rule 19 of the Rules (see [Animals and Birds Act](#) in [Appendix I: Reference materials: Legislations](#)). The information in the annual report must include the following:

a. Assurance

- i. Assure that professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anaesthetics, analgesics, and tranquillising drugs were maintained.
- ii. Assure that each PI has considered alternatives to procedures that cause pain or distress to animals.
- iii. Assure that a mechanism is in place to monitor and resolve non-compliances to the NACLAR Guidelines, and that it has required that exceptions to the NACLAR Guidelines be specified and explained by PIs and approved by the IACUC.
 - a) A summary of all such exceptions including a brief explanation must be attached to the annual report.

b. Background information and statistics

- i. The composition of the IACUC.
- ii. The name of the AV and whether employed on a full-time or part-time basis.
- iii. The location of all facilities where animals are housed, used or held for scientific activities.
- iv. The common names and the numbers of animals used for scientific activities at each facility involving no more than momentary or slight pain or distress and no use of pain-relieving drugs. Routine procedures (e.g. injections, tattooing, blood sampling), should be reported with this group.
- v. The common names and the numbers of animals used for scientific activities at each facility involving accompanying pain or distress to the animals and for which appropriate anaesthetics, analgesic, or tranquillising drugs were used.

- vi. The common names and the numbers of animals used for scientific activities at each facility involving accompanying pain or distress to the animals and for which the use of appropriate anaesthetics, analgesic, or tranquillising drugs would have adversely affected the procedures, results, or interpretation of the research, experiments, surgery, or tests.
 - a) An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used must be attached to the annual report.
- vii. The common names and the numbers of animals being bred, conditioned, or held for scientific activities at each facility but not yet used for such purposes, as of Dec. 31.
- viii. The dates of reviews (e.g. protocol reviews, reportable incidents, etc.) and inspections by the IACUC.
- ix. Significant deficiencies identified in the annual programme review and facility inspections by the IACUC, and whether the actions taken to correct these deficiencies were as planned and scheduled in the IACUC reports.
 - a) A significant deficiency is one that is or may be a threat to the health and safety of humans and/or animals.

II.9.3.2 Institutions should ensure they are aware of NParks/AVS' specific animal number reporting criteria.

II.10 Institutional Animal Care and Use Committee (IACUC)

II.10.1 Further and detailed guidelines on the operation of the IACUC and the standards that the IACUC should set are outlined in [Section III: The IACUC](#).

II.10.2 The minimum training requirements for IACUC members and other training that is recommended are set out in [IV.6](#).

II.11 Responsibilities of Principal Investigators (PIs)

II.11.1 General

II.11.1.1 PIs must act in accordance with the NACLAR Guidelines.

II.11.1.2 The responsibility of PIs extends from the time the animal is allocated to the approved protocol to the disposal of the animal.

II.11.1.3 PIs are responsible for the conduct of all personnel, students, visitors etc. working on their protocol, and must ensure that all have read and understood the protocol, are appropriately trained and supervised, and follow the protocol meticulously.

II.11.1.4 PIs must consult with the AV whenever there are adverse or unexpected outcomes.

II.11.2 IACUC approval

II.11.2.1 Before any protocol begins, PIs must submit a proposal to the IACUC.

II.11.2.2 PIs, and those working on their protocol, must not begin work on a protocol or amendments to a protocol before written IACUC approval is obtained, and must adhere to the approved protocol and any requirements of the IACUC.

II.11.2.3 PIs may obtain and hold for acclimatisation or adaptation species which are not normally readily available, prior to formal IACUC approval of their protocol. However, they must obtain IACUC approval for these activities first, by submitting to the IACUC for consideration a document specifying the intention to obtain and hold these species, with appropriate justification and a timetable.

II.11.2.4 PIs should inform the IACUC in writing when each protocol is completed or discontinued; and the outcome of each protocol.

II.11.3 Planning protocols

II.11.3.1 Choice of Animal – Refer to [II.4.4.1](#).

II.11.3.2 Monitoring

- a. PIs should ensure that all animals are observed daily (or more frequently if circumstances require it) to assess their health and welfare.
- b. PIs should ensure that satisfactory arrangements are made for contacting them, and other responsible persons, in the event of emergencies.

II.11.3.3 Record-keeping

- a. PIs should ensure that experimental records and breeding records are kept and maintained for at least three years from the completion of the protocol.

II.11.3.4 Consultation

- a. The AV must be consulted in the planning of any practice or procedure that can cause pain to animals, including:
 - i. use of tranquillisers, analgesics, anaesthetics, and paralytics;
 - ii. pre-surgical, surgical and post-surgical care;
 - iii. the withholding of tranquillisers, anaesthesia, analgesia, or euthanasia when scientifically necessary.
- b. The AV must be consulted on the use of appropriate euthanasia methods.

II.11.3.5 Checklist

- a. When planning is completed, the PI should re-check the protocol to ensure that the following points have been adequately addressed:
 - i. Is the protocol justified ethically and scientifically?
 - ii. Can the aims be achieved without using animals?
 - iii. Are there any alternative methods that could be included that would reduce the number of animals used?
 - iv. Are suitable holding facilities and competent staff available?

- v. Has the most appropriate species of animal been selected?
- vi. Are the experiments designed so that statistically valid results can be obtained, or the educational objectives can be achieved, using the minimum necessary number of animals?
- vii. If the scientific activity could cause the animals any pain or distress, what will be done to minimise or avoid this?
- viii. Do all project personnel have the skills and experience to perform these procedures?
- ix. Does this project involve students, and will they be appropriately supervised?
- x. What arrangements will be made to monitor the animals adequately, in terms of their general health and welfare and response to manipulation?
- xi. If any of the experiments have been performed previously, why should they be repeated?
- xii. Are there any permits that must be obtained for the importation, capture, breeding, use, euthanasia or release of the animals?
- xiii. If multiple survival surgeries are proposed, are they scientifically justified? Refer to [III.4.20](#).

II.11.4 Conduct of experiments

II.11.4.1 Limiting pain and distress – Refer to [II.4.4](#).

- a. The PI should anticipate any potentially adverse effects of a manipulation on the animal, and take all possible steps to avoid or minimise the pain and distress. These steps should include:
 - i. choosing the most appropriate and humane method for the conduct of the experiment;
 - ii. ensuring the technical skills and competence of all persons involved in animal care and use are appropriate;
 - iii. use of pre-emptive analgesia when pain is anticipated;
 - iv. ensuring that animals are adequately monitored for evidence of pain and distress;

- v. developing a plan to manage any adverse effects of a manipulation;
 - vi. acting promptly to alleviate pain and distress;
 - vii. using anaesthetic, analgesic and tranquillising agents appropriate to the species and the experimental purposes;
 - viii. developing humane and experimental endpoints that minimise pain and distress;
 - ix. conducting projects over the shortest time practicable; and
 - x. using appropriate methods of euthanasia.
- b. Distress can often be avoided or minimised by non-pharmacological means. For example, before an experiment begins, animals should be appropriately habituated to the experimental environment and procedures, and familiarised with the research and animal care staff.
 - c. The monitoring of animals during and after experiments must at all times be adequate to prevent the occurrence of pain or distress, or allow prompt alleviation.
 - d. Appropriate nursing procedures to minimise pain and distress and promote the well-being of the animals should be provided.
 - e. If animals develop signs of severe pain or distress despite the precautions outlined above, they should have the pain or distress alleviated promptly or be euthanised without delay. The AV, or his/her designee, should be informed immediately. Alleviation of such pain or distress takes precedence over continuing or finishing the experiment. If in doubt, PIs must consult with the AV, or his/her designee, before continuing an experiment.
 - f. Unexpected deaths must be reported to the AV, or his/her designee, to be properly investigated to determine the cause and initiate remedial action. If the unexpected deaths are due to a manipulation or a set of manipulations, the particular manipulation or set of manipulations should be refined or mitigated to prevent such occurrences.

II.11.4.2 Animal welfare monitoring of pain or distress

- a. PIs and those working on the protocol should be familiar with the normal behaviour patterns of the animal species they are working with, be knowledgeable of signs of pain or distress specific to that species, and must monitor the animals for these signs.

- b. Deviations from normal behaviour patterns are often the first indications that animals are experiencing pain or distress. Any changes in patterns of sleeping, feeding, drinking, grooming, exploratory behaviour, performance in learning or discriminatory tasks, reproduction or social behaviour should be reported to the AV or his/her designee.
- c. Animals must be monitored appropriately for clinical signs of acute pain or distress. Refer to [Appendix V: Signs of Animal Pain and Distress](#) for details.
- d. Animal welfare score sheets can be useful for documenting the observations and deciding when humane endpoints have been reached. Refer to NAEAC in [Appendix I: Section II: General](#) for sample monitoring sheets.

II.11.5 Endpoints

- II.11.5.1 Experimental endpoint: When preparing a protocol, the PI must establish an experimental endpoint at which the scientific aims and objectives of the study have been reached.
- II.11.5.2 Humane endpoints: The PI must also establish humane endpoints, i.e. provide criteria used to determine the point at which pain or distress in an experimental animal will be prevented, terminated, or relieved prior to the planned experimental endpoint.
 - a. Refer to [CCAC](#) in [Appendix I: Section II: General](#) for more details on humane endpoints.
- II.11.5.3 Death as an endpoint: Unless there is strong scientific justification for death as an endpoint (refer to [III.4.4.4](#) and [CCAC](#) in [Appendix I: Section II: General](#)), death as an endpoint must be avoided.
 - a. If death as an endpoint must be used, and is approved by the IACUC, the PI must ensure that the animal's distress or pain is minimised, including the appropriate use of sedation, analgesia, anaesthesia or other interventions.

II.11.6 Repeated use of animals in experiments

- II.11.6.1 Individual animals should not be used in more than one experiment, either in the same protocol or different protocols, unless approved by the IACUC.
- II.11.6.2 When approving protocols involving the re-use of animals, the IACUC should be satisfied of the following:

- a. none of the procedures are likely to cause the animals pain or distress;
or
- b. the second and subsequent studies are non-survival studies, or produce little or no pain or biological stress to the animals (e.g. modifying diet, taking a succession of blood samples, repeated non-invasive recording procedures).

II.11.6.3 Animals that are used in more than one experiment must be permitted to recover fully from the first experiment before the subsequent experiment is performed.

II.11.7 Duration of experiments

II.11.7.1 Experimental duration should be limited to that sufficient to achieve the objective of the experiment.

II.11.7.2 Experiments, particularly those involving any pain or distress, should be as brief as practicable.

II.11.8 Handling and restraining animals – Refer to [III.4.10](#)

II.11.8.1 Animals should be handled by competent individuals trained in methods that cause minimal distress and injury.

II.11.8.2 The use of restraint devices is sometimes essential for the welfare of the animal and safety of the handler.

II.11.8.3 Restraint devices should be used to the minimum extent, for the minimum period required to accomplish the purpose of the experiment and be appropriate for the animal. Periods of prolonged restraint should be avoided.

- a. The IACUC must determine the maximum allowable period of restraint considering the animals' biological needs, including their behavioural requirements and their needs for appropriate exercise and rest.
- b. In addition to PI/researcher monitoring, these animals should be monitored regularly by the AV or his/her designee. If any ill effects are apparent, the animal should be removed from the restraint device or the method modified.

II.11.9 Euthanasia – Refer to [III.4.3](#)

II.11.9.1 The prevailing AVMA Guidelines for the Euthanasia of Animals (see [AVMA Panel on Euthanasia](#) in [Appendix I: Section II: Euthanasia](#)) must be adhered to.

- II.11.9.2 When it is necessary to euthanise animals, humane procedures must be used. These procedures should avoid distress, be reliable and produce rapid loss of consciousness while minimising pain, until death occurs.
- II.11.9.3 The choice of a method of euthanasia depends on the species, age, availability of restraint, and skill of the individual performing the euthanasia, and should be consistent with the research goals.
- II.11.9.4 The appropriate means and materials must be readily at hand.
- II.11.9.5 The procedures must be performed by competent persons.
- II.11.9.6 Animals should be euthanised in a quiet, clean environment, and away from other animals.
- II.11.9.7 Criteria to ensure death must be detailed in the protocol, including a secondary method of euthanasia, where appropriate.
- II.11.9.8 Dependent neonates of animals to be euthanised must also be euthanised or provisions made for their care.
- II.11.9.9 When fertilised eggs are used, the method of disposal must ensure the death of the embryo.

II.11.10 Protocols involving surgery – Refer to [III.4.19](#)

II.11.10.1 Pre-operative planning

- a. Surgical success can be improved by careful attention to the following depending on the context of the study:
 - i. The use of healthy, disease-free animals.
 - ii. Pre-operative physical examination can often identify potential problems and should be carried out.
 - iii. For some species, a period of pre-surgical fasting should be considered to minimise complications of anaesthetic administration. Plans should be developed in conjunction with advice from the AV as to the appropriate period of pre-surgical fasting for the given species and procedure.
 - iv. Antibiotic administration should only be given when appropriate and should not be used as a replacement for aseptic technique.

- v. Surgical time can frequently be reduced by practice on cadavers. This enables researchers to familiarise themselves with anatomical landmarks and streamline the experimental surgical procedures, thereby reducing the duration of anaesthesia and post-operative recovery.
- vi. The use of imaging (e.g. MRI, CT, radiography, ultrasonography, PET-CT) may be useful to guide surgical planning and to provide intra-operative image guidance.
- vii. Pre-operative analgesia should be routinely used. Such pre-emptive use of analgesics can reduce the quantities of general anaesthetic agents required and prevent the induction of sensitisation of the central nervous system. Post-operative pain is best managed by pre-emptive analgesic administration, followed by additional analgesics post-operatively. The specific analgesic compounds selected and the duration of post-operative administration should be appropriate for the invasiveness of the procedure or surgery.
- viii. Use of aseptic technique.
- ix. The use of appropriate surgical facilities. Refer to [III.4.19.5-6](#).

II.11.10.2 Surgery

- a. Surgical procedures should be carried out under appropriate local or general anaesthesia and depth of anaesthesia must be monitored periodically during the procedure.
- b. Adequate warming should be provided during the procedure. Alternatives to heat lamps and electric heating pads should be considered (examples include circulating water blankets, disposable air activated warmers for rodents, hot air blankets).
- c. The choice and administration of anaesthetic, analgesic and tranquilising agents should be suitable for the species and appropriate for the purpose of the experiment.
- d. Anaesthesia and surgery must be performed by competent staff with appropriate training and experience.
- e. When more than one surgical procedure is to be performed, the animal must have recovered to good general health before the next procedure. Every effort should be made to reduce the total number of procedures and the IACUC must be informed specifically, and provide approval, for more than one procedure.

- f. Aseptic technique must be used for all survival surgery, and is recommended for non-survival procedures especially for surgeries of long duration. Aseptic technique includes appropriate preparation of the surgical field, use of sterilised instruments, sterile surgical gloves and gowns, head cover, and surgical masks.

II.11.10.3 Post-operative care

- a. Attention to pain relief is paramount in post-operative care.
- b. PIs must ensure that adequate monitoring, treatment and care of post-operative animals are provided. Animals should always be monitored closely to ensure recovery from anaesthesia and individual response to analgesic therapy. Adjustments in analgesic dosage should be made to ensure animal comfort, where needed. Analgesic use must be documented.
- c. The comfort and welfare of animals must be promoted throughout the post-operative period. Attention should be given to warmth, hygiene, fluid and food intake, and wound care.
- d. Care should be taken that animals recovering from anaesthesia are managed to prevent injury.
- e. Regular observation of surgical wounds is essential.
- f. Any post-operative animal observed to be in a state of severe pain or distress that cannot be alleviated quickly must be euthanised without delay.

II.11.11 Implanted devices

- II.11.11.1 PIs should be aware of the need for strict attention to aseptic technique when devices are surgically implanted.

II.11.12 Neuromuscular paralysis

- II.11.12.1 Neuromuscular blocking agents must not be used without adequate general anaesthesia or an appropriate surgical procedure that eliminates sensory awareness, and must be justified.
- II.11.12.2 Special care must be taken to ensure the maintenance of an adequate plane of anaesthesia, until the effects of the neuromuscular blocking agent have fully subsided.
- II.11.12.3 Continuous or frequent intermittent monitoring of physiological variables such as heart rate, blood pressure, oxygen saturation, pupil size and brain activity must be conducted.

- a. Care is required to ensure that drugs used in the experiments do not interfere with this monitoring.
- b. Respiration and corneal and flexor withdrawal reflexes must not be used to judge the level of anaesthesia when neuromuscular blocking agents are used.

II.11.13 Electro-immobilisation

- II.11.13.1 Electro-immobilisation must not be used as an alternative to analgesia or anaesthesia.

II.11.14 Animal models of disease

- II.11.14.1 The scientific validity of an animal model of human or animal disease rests in part on how closely it resembles a particular human or animal disease. An animal should be used only if the disease in the animal can serve as a reliable model for investigation into the human or animal disease.
- II.11.14.2 It must be assumed, unless there is contrary evidence, that the attendant pain and distress of the human or animal disease will also occur in the animal. The PI must therefore take special care to ensure that any pain or distress is minimised and the IACUC is informed of the potential effects of the disease on the animals.
- II.11.14.3 PIs must not allow the experiments to proceed to a painful, distressful or lingering death of animals unless no other experimental endpoint is feasible, and has been approved by the IACUC. Refer to [II.11.5](#).

II.11.15 Modifying animal behaviour

- II.11.15.1 Procedures used to modify an animal's behaviour or to induce it to perform specific tasks depend on motivating the animal. Positive reinforcement should be used whenever possible.
- II.11.15.2 If an inducement is needed to modify behaviour, it should be as mild as possible. Severe water, food, social or sensory deprivation should not be used. Painful or noxious stimuli should be limited and must be used for the minimum time necessary.

II.11.16 Toxicological experiments – Refer to [III.4.14](#)

II.11.17 Experimental manipulation of animals' genetic material - Refer to [II.4.8](#)

- II.11.17.1 Such experiments must be in accordance with the Singapore Biosafety Guidelines for Research on GMOs as set out by the Genetic Modification Advisory Committee (GMAC) (see [GMAC](#) in [Appendix I: Section II: General](#)).
- II.11.17.2 All proposals to manipulate the genetic material of animals, their germ cells or embryos must be submitted to the IACUC for approval.
- II.11.17.3 GMOs are sometimes accompanied by unintended and unpredicted alterations that adversely affect animal well-being. PIs need to establish a plan to monitor for and address unanticipated adverse outcomes for GM animals.
- II.11.17.4 PIs must inform the IACUC of the known potential adverse effects to the well-being of the animals and their offspring, and detail if anything can or will be done to ameliorate such adverse effects, and what endpoints will be used to determine when an animal will be euthanised.
- II.11.17.5 There should be a plan for systematic characterisation of phenotypes to facilitate assessment of their possible utility and timely decisions on disposition or retention.
- II.11.17.6 The clinical status of animals in which the genetic material has been manipulated experimentally must be monitored for unusual or unexpected adverse effects, and such effects reported to the IACUC.
- II.11.17.7 PIs must:
 - a. not generate a new animal line using genetic modification if a similar, suitable animal model is available to the PI or a relevant in vitro method can be used to achieve the aims of the project;
 - b. ensure that the IACUC is kept informed about the impact of the genotype on animal well-being as well as mortality, morbidity and population health. Procedures used for creating and breeding these animals must be regarded as part of a project and must be included in the protocol application to the IACUC;
 - c. use methods to support and safeguard the well-being of the animals involved;
 - d. maintain records of the number of animals used to create and maintain the new animal line, and the lineage and clinical health of the animals; and
 - e. ensure that reports are provided to the IACUC, including:

- i. regular monitoring reports of a new animal line at a frequency to be determined by the IACUC; and
- ii. a final report on the generation of the new animal line.

II.11.17.8 Cloning of animals may or may not involve genetic modification. However, as cloning by the technique of somatic nuclear transfer may be associated with unexpected adverse events, the PI should inform the IACUC of any potential side-effects that may impact negatively on the welfare of the parent animal or its offspring, and of the means that will be used to deal with such eventualities. Details of monitoring for unexpected adverse effects arising from the genetic modification must be provided.

II.11.18 Experimental induction of neoplasia/tumour studies – [Refer to III.4.11](#)

II.11.19 Withholding food or water

II.11.19.1 Experiments involving the withholding or severe restriction of food or water should produce no enduring detrimental effect on the animals, and must be kept to a minimum. Amount of fluid/food intake and/or body weight must be monitored, recorded and maintained within the limits approved by the IACUC. Body weight records and fluid/food intake records should remain with the animals in the holding facility.

II.11.20 Foetal experimentation

II.11.20.1 Unless there is specific evidence to the contrary, PIs must assume fetuses have the same requirements for anaesthesia and analgesia as adult animals of the species.

II.11.20.2 Eggs must be destroyed before hatching, unless hatching is a requirement of the experiment. The IACUC must approve the arrangements made for hatchlings.

II.11.21 Research on pain mechanisms and the relief of pain

II.11.21.1 For experiments in which unanaesthetised animals are to be subjected to stimuli designed to produce pain, or when pain is to be inflicted on animals as part of normal management, PIs must satisfy the IACUC that their choice of the measurement of pain is appropriate.

II.11.21.2 PIs must:

- a. ensure that the pain stimuli are limited at all times to the minimum pain necessary for the experiment; and

- b. provide treatment for the relief of pain, allow self-administration of analgesics or escape from repetitive, painful stimuli, whenever possible.

II.11.22 Animal health and welfare research

II.11.22.1 When studying ways of improving the health and welfare of animals, PIs may need to design experiments that replicate conditions such as injury, trauma, nutritional disorder, physical exertion, disease or environmental stress. When such experiments are necessary, the PI must ensure that:

- a. the principal aim of the protocol is to improve animal health or welfare;
- b. alternative methods, such as the use of animals naturally inflicted with the condition, are not possible; and
- c. all possible steps are taken to minimise any pain or distress.

II.11.23 Field studies – Refer to [III.4.17](#)

II.11.24 Training for Principal Investigators

II.11.24.1 The minimum training requirements for PIs and other training that is recommended are set out in [Section IV: Training](#).

II.12 Responsibilities of Instructors

II.12.1 Teaching at tertiary levels

II.12.1.1 When animals are being used to achieve educational objectives, the course instructor must:

- a. ensure that the care and use of the animals complies with the NACLAR Guidelines;
- b. have relevant training and qualifications;
- c. consider whether alternative teaching methods can be used;
- d. obtain prior IACUC approval (refer to [III.4.18](#));
- e. instruct students appropriately in the care and use of animals before the students participate in experiments with live animals;
- f. ensure that there is close, competent supervision of all students;
- g. allow students to anaesthetise animals or carry out surgery only if it is essential for their training; and
- h. ensure the animals be euthanised, if required.

II.12.1.2 No student should be forced to use an animal against his/her will.

SECTION III: THE IACUC

III.1 IACUC Functions

III.1.1 Main function

- III.1.1.1 The IACUC is responsible for the oversight and evaluation of all aspects of the institution's animal care and use programme and advises the IO on the steps required to maintain animal research facilities and programmes such that they conform to the NACLAR Guidelines and other relevant laws and guidelines.

III.1.2 Specific functions:

- III.1.2.1 to review, at least once every 12 months, all programmes for the care and use of animals carried out in the research facility, using the guidelines as a basis of evaluation;
- III.1.2.2 to inspect the research facility, including the housing, holding and satellite facilities; and procedure areas for animals, at least once annually, using the guidelines as a basis of evaluation;
- III.1.2.3 to prepare reports of the evaluations conducted by the committee in accordance with such requirements as may be set out in the guidelines and submit the reports directly to the IO;
- III.1.2.4 to review and investigate any concern, complaint or report of non-compliance with any guideline involving the care and use of any animal at the research facility;
- III.1.2.5 to make recommendations to the IO regarding any aspect of the animal programme, facilities or personnel training at the research facility;
- III.1.2.6 to review, approve, require modifications in, withhold approval of or reject any proposed protocol, or any proposed significant change in any ongoing protocol, relating to the use of any animal for any scientific purpose;
- III.1.2.7 to withdraw approval for or suspend any protocol involving any research animal if that protocol is not being conducted in accordance with the guidelines or with the description thereof provided by the PI and approved by the IACUC;
- III.1.2.8 to conduct continuing reviews of activities involving the use of any animal for any scientific purpose, including any approved protocol of long duration and any long-term continual use of any individual animal or such purpose, at appropriate intervals but at least once a year;

- III.1.2.9 to determine the best means of conducting an evaluation of the care and use of animals, provided that no member of the IACUC wishing to participate in any evaluation is excluded;
- III.1.2.10 to authorise the treatment or humane killing of any animal for any scientific purpose;
- III.1.2.11 to maintain a register of approved protocols; and
- III.1.2.12 to perform all duties as required under the guidelines.

III.1.3 Authority of the IACUC

- III.1.3.1 The IACUC derives its authority from the institution, acting through the IO.
- III.1.3.2 The institution, acting through the IO, appoints the members of the IACUC; provides resources to the IACUC needed for its operation; and, enforces the IACUC recommendations.
- III.1.3.3 The IACUC has authority to conduct reviews of the animal care and use programme and inspections of animal care and use facility(ies). Refer to [III.1.7](#).
- III.1.3.4 The programme reviews and facility inspections, conducted by the IACUC, inform the IO of the institution's compliance; establishes plans and schedules for correcting deficiencies; and makes recommendations regarding any aspect of the institution's animal programme, facilities, or personnel training.
 - a. This approach of "enforced self-regulation" requires that the IACUC has the full support of the institution.
- III.1.3.5 The IACUC has the authority to approve protocols independent of the IO.
- III.1.3.6 The IO cannot overrule an IACUC decision to withhold approval of a protocol.
- III.1.3.7 However, if an IACUC approves a protocol, the institution is not required or obligated to conduct the research activity.
- III.1.3.8 The IACUC is authorised to suspend approved protocols and/or any activities involving animals in accordance with the NACLAR Guidelines. Refer to [III.6.6.3](#).
- III.1.3.9 The IACUC must report to the IO the reasons for suspension, recommend appropriate corrective action, and report actions taken.

III.1.4 Membership composition and qualifications

- III.1.4.1 An IACUC must comprise at least five persons, with at least one person representing each of the four categories listed below. One person cannot fill more than one category. The different categories are intended to provide the IACUC with well-balanced views and decisions. The IO cannot be appointed as a member of an IACUC given that the IACUC reports to the institution, acting through the IO.
- a. The institution's AV, who is a licensed veterinarian with training and/or experience in laboratory animal science and medicine and who has expertise in the routine care of the species of animals worked with in the research facility. The AV must become familiar with the care of new or unusual species of animals proposed for use at the institution.
 - b. A scientist with appropriate experience in the use of animals for scientific purposes.
 - c. A person who (i) is not affiliated with the institution; (ii) is not a member of the immediate family of any person who is affiliated with the institution; and (iii) is not a user of any animal for any scientific purpose.
 - i. To clarify, payment to reimburse reasonable transport costs and related expenses is permissible without jeopardising a member's non-affiliated status.
 - d. A person whose primary concerns or interests are in non-scientific areas (e.g. ethicist, lawyer, clergy, clerical or administrative staff). The non-scientist may be affiliated with the institution.
- III.1.4.2 To come up with five or more members, there can be more than one person from any one of the four categories set out in [III.1.4.1.a-d](#).
- III.1.4.3 In order not to influence IACUC decisions, no more than three members are permitted from the same department or unit within the institution.
- III.1.4.4 Alternate members may be appointed to the IACUC to represent primary IACUC members.
- a. An IACUC member and his/her alternate(s) may not count toward a quorum at the same time or act in an official member capacity at the same time.
 - b. Alternates should receive training identical to the training provided to primary IACUC members.

III.1.4.5 The Chair of the IACUC is to be appointed by the IO from amongst the members of IACUC.

- a. The Chair should either hold a senior position in the institution, or if an external appointee be given a commitment by the institution to provide the necessary support and authority to carry out his/her role.
- b. The Chair must be supported by the institution and IO, so that the Chair is able to speak candidly and make decisions without fear of reprisals.
- c. The AV should not take the position as Chair.

III.1.4.6 The institution may temporarily assign an Acting Chair from the IACUC members during the absence or abstention of the IACUC Chair.

III.1.5 Conflict of interest

III.1.5.1 When an IACUC member has a conflict of interest (e.g. named in the protocol, or involved in a potentially competing research programme) the member must disclose the nature of the conflict at the meeting of the IACUC and must not participate in the IACUC review or deliberation, and must not vote on the protocol. This applies to any IACUC action related to the protocol, including but not limited to: review of the protocol, investigation of animal welfare concerns and other non-compliances.

III.1.5.2 If a PI submitting a protocol believes that an IACUC member has a potential conflict of interest, the PI may request that the member be excluded from the decision-making pertaining to the approval of the protocol.

III.1.6 Quorum and voting requirements

III.1.6.1 IACUC actions that require a quorum are:

- a. approval of a protocol not assigned to designated member review (DMR) (refer to [III.3.2.3](#)),
- b. change of policy,
- c. approval of exceptions to the NAELAR Guidelines, and
- d. suspension/withdrawal of approval of a protocol.

III.1.6.2 “Quorum” is defined as 50% or more of the members of the IACUC and must include the AV or the AV’s alternate IACUC member, and at least, one representative from category c. or d. as described in [III.1.4.1](#). Therefore:

- a. A protocol is approved if a quorum is present at a convened meeting, and if a majority of the quorum votes in favour.
- b. To suspend an activity, or withdraw approval for a protocol, the IACUC must review the matter at a convened meeting with a quorum present and the suspension/withdrawal of approval must be approved by a majority of the quorum present.

III.1.6.3 For the avoidance of doubt:

- a. Members who are disqualified due to a conflict of interest must not contribute to a quorum or vote.
- b. Abstentions from voting do not alter the quorum or change the number of votes required.
 - i. For example: If an IACUC has eleven members, at least six members who are not disqualified must be present at a convened meeting to constitute a quorum and approval of a protocol would require a minimum of four votes for approval, whether or not there were abstentions.
 - ii. In the event of a tie in the number of votes, the Chair shall have the casting vote.

III.1.7 Programme review and facility inspection functions

III.1.7.1 The IACUC must review, at least once in 12 months, the institution's programme for the care and use of animals:

- a. The programme review:
 - i. provides an ongoing mechanism for ensuring that the institution maintains compliance with applicable animal care and use policies, guidelines and laws;
 - ii. serves as an opportunity for constructive interaction and education for the animal care personnel, research staff, and IACUC members; and
 - iii. helps an institution prepare for subsequent visits by external evaluators, such as AAALAC International.

III.1.7.2 The IACUC must inspect, at least once in 12 months, all of the institution's animal care and use areas.

III.1.7.3 The annual facility inspection and the programme review must be conducted separately and at different times.

- a. To enhance IACUC oversight of the animal care and use programme and to enable sufficient time gap between these complementary activities, there should be a 3 to 7-month separation period between a facility inspection and a programme review.

III.1.7.4 The IACUC may determine the best means of conducting an evaluation of the programme and inspection of the facilities. The IACUC may invite ad hoc consultants to assist in conducting the evaluation. However, the IACUC remains responsible for the evaluations and reports.

III.1.7.5 The IACUC must prepare reports of the IACUC facility inspection and programme review and submit them to the IO in a timely manner.

III.1.7.6 The IACUC must ensure that all deficiencies identified during the programme review and facility inspections are corrected as specified in the report and in the timeframe stipulated.

III.1.8 Institutional responsibility for animal welfare

III.1.8.1 Assuring laboratory animal welfare and a high-quality animal care and use programme, necessitates a partnership between the IO, the IACUC, the AV and PIs.

- a. Ultimately, accountability for assuring humane care and use of animals resides with the IO, but this may only be achieved when participants - the PIs and their research staff, the veterinary staff, animal caretakers and technicians, and the IACUC - all contribute to a shared goal.

III.1.8.2 Each institution should have in place a framework with appropriate resources for an animal care and use programme that is managed in accordance with the NACLAR Guidelines.

- a. Institutions that function effectively have simple, clear and direct lines of responsibility and corresponding authority.

III.1.9 Additional human resources

III.1.9.1 IACUC Secretariat

- a. It is advisable that an IACUC, especially for large institutions, have dedicated staff, i.e. secretariat.

- b. The IACUC secretariat must have knowledge of applicable regulations and guidelines; must undergo the same training that IACUC members receive (refer to [IV.6](#)); and must maintain all official records, as appropriate.

III.1.9.2 Ad hoc Consultants

- a. Ad hoc consultants can be sourced by the IACUC in special cases where specific professional advice is needed.

III.1.9.3 Training of IACUC members and staff – Refer to [IV.6](#).

III.2 Oversight of the Animal Care and Use Programme

III.2.1 Conducting programme reviews

III.2.1.1 Key aspects of an animal care and use programme that should be emphasised in the annual evaluation include, but are not limited to:

- a. record-keeping practices;
- b. occupational health and safety for the animal care and use programme;
- c. disaster plan and emergency preparedness;
- d. housing, environment and management;
- e. investigating and reporting concerns regarding animal welfare;
- f. quality of the veterinary care program;
- g. IACUC membership, functions and procedures, including protocol review and evaluation of training programmes;
- h. ongoing post-approval monitoring;
- i. personnel qualifications and training;
- j. institutional policies and responsibilities, including availability of adequate resources, appropriate communication between the IO, the IACUC and AV, and formal agreements for inter-institutional collaborations;
- k. methods employed to meet reporting requirements; and
- l. personnel security, including preventative measures such as pre-employment screening and physical and information technology security.

III.2.1.2 Specific procedures to accomplish programme evaluations may include presentations by specific individuals (AV, occupational health and safety representative etc.) and review of policies and SOPs.

III.2.1.3 The United States National Institutes of Health (NIH) Office of Laboratory Animal Welfare (OLAW) website provides helpful checklists (see [OLAW](#) in [Appendix I: Section III: The IACUC](#)).

III.2.2 Facility inspections

III.2.2.1 All animal care and use facilities must be inspected. The OLAW checklist may be referred to as a helpful resource (see [OLAW](#) in [Appendix I: Section III: The IACUC](#)). The following should be included in the inspection:

- a. housing facilities,
- b. satellite facilities,
- c. procedure areas, and
- d. the institution's animal transport vehicle(s).

III.2.2.2 Staffing and scheduling the facility inspections

- a. Inspections may be accomplished by assigning specific facilities to teams, each of which must consist of at least two IACUC members, in addition to the AV or a designated alternate, for a total of at least three inspection team members.
 - i. No IACUC member should be excluded should he/she wish to participate in any inspection.
 - ii. The inspection team should have a thorough working knowledge of the NACLAR Guidelines to evaluate the facilities.
- b. The IACUC may determine whether the supervisory personnel of various facilities should be notified of the date and time of inspection.
 - i. Advance notification allows individuals to be available to answer questions;
 - ii. an unannounced inspection may better show the facility during usual operations, but also may result in having to be rescheduled if key individuals are not available.

- c. While the inspection of each facility must be conducted annually there is no requirement for all facilities to be inspected at the same time.

III.2.3 Performing facility inspections

III.2.3.1 Aspects to be covered during inspections include, but are not limited to, the following:

- a. location, construction, environmental control (temperature, humidity, ventilation, air quality, vibration, noise), security and access control;
- b. primary enclosures;
- c. environmental enrichment, behavioural and social management;
- d. food, water, bedding;
- e. water quality (aquatics);
- f. sanitation;
- g. waste disposal;
- h. pest control;
- i. emergency, weekend and holiday animal care;
- j. animal identification;
- k. record keeping;
- l. breeding genetics and nomenclature;
- m. storage;
- n. cage wash areas;
- o. aseptic surgery areas;
- p. special facilities, including areas used for procedures, rodent surgery, imaging, whole body irradiation, hazardous agent containment, and behavioural testing;
- q. use of controlled and/or expired drugs;

- r. occupational safety and health concerns (e.g. first aid box, NHP exposure kit, eye wash stations, emergency shower, emergency lighting, smoke detectors, fire alarms);
- s. staff training (e.g. staff compliance with SOPs, correct use of personal protective equipment [PPE]);
- t. knowledge of applicable rules and regulations (e.g. observing activities and questioning personnel, checking records); and
- u. building integrity (e.g. floors, walls, ceilings).

III.2.3.2 Adherence to the following recommendations will assist the IACUC in performing inspections:

- a. An updated list of all facilities, including satellite facilities and procedure areas, and vehicles must be maintained by the IACUC secretariat.
 - i. This list should include the contact person, species and procedures (survival or non-survival) approved by the IACUC to be performed in each satellite and procedure area.
 - ii. Protocols must specify all locations where all animal procedures will be performed.
- b. A list of all deficiencies identified during the previous IACUC inspection, NParks/AVS inspection, and other inspections, should be maintained and provided to the IACUC inspectors. This list should include the assigned correction dates, plans for corrections, and the status of those corrections.
- c. For facilities with multiple rooms, a floor plan can assist the IACUC inspection team.
- d. Deficiencies identified during the IACUC inspection should be discussed, whenever possible, with the person in charge of the facility to ensure that the team's perception of the situation is accurate.
 - i. In some cases, an apparent deficiency may be due to an approved aspect of a protocol in progress (e.g. withholding food prior to surgery).
- e. Use of a checklist provides consistency and helps document the completeness of the inspection.

III.2.4 Documentation

- III.2.4.1 There must be written reports of the annual programme review and annual facility inspections.
- III.2.4.2 The reports must be approved and signed by the majority of the IACUC.
- III.2.4.3 The reports must describe:
 - a. significant changes in the animal care and use programme, since the last report,
 - b. deficiencies identified, and
 - i. Deficiencies must be designated by the IACUC as either minor or significant.
 - ii. A significant deficiency is defined as a situation that is or may be a threat to animal health, well-being or welfare; or human/animal safety.
 - iii. A reasonable and specific plan and date for correction must be included in the final report, for both minor and significant deficiencies.
 - a) The individual responsible for corrections should be consulted when appropriate to ensure that the plan is realistic.
 - c. suggestions for improvement to the animal care and use programme.
- III.2.4.4 The report must indicate whether or not any minority views were filed, and minority views must be included in the report.
- III.2.4.5 A copy of the report is provided to the IO and must be kept for a minimum of three years. The report should be delivered in person to the IO in order to emphasise the findings and plans for action.
- III.2.4.6 A failure to ensure the report contains the necessary details and/or is kept for the stipulated time frame could lead to sanction under the [Animals and Birds \(Care and Use of Animals for Scientific Purposes\) Rules](#).

III.3 **Review of Protocols**

III.3.1 General

- III.3.1.1 The IACUC is responsible for overseeing and evaluating all aspects of animal care and use, and is charged with reviewing proposed protocols that involve animals.

- III.3.1.2 In its review of protocols, the IACUC's primary goal should be to facilitate compliance with applicable guidelines, laws, regulations, rules and policies consistent with optimal animal welfare and the performance of appropriate and productive scientific endeavours.
- a. The IACUC must influence protocols to comply with the *Human Biomedical Research Act (HBRA) (Act 29 of 2015)* (see [HBRA](#) in [Appendix I: Reference Materials: Legislations](#)), when it relates to human-animal combinations.
 - i. Research protocols falling under the [HBRA 3rd Schedule \(Prohibited Research\)](#) must be rejected. For example, breeding of animals that have had any kind of human pluripotent stem cells introduced into them is prohibited.
 - ii. Research protocols falling under [HBRA 4th schedule \(Restricted Research\)](#) must be submitted for review and approval to the Ministry of Health (MOH). For example, introduction of human pluripotent stem cells into a living postnatal animal must be approved by MOH.
 - iii. Some human-animal combinations fall outside the scope of HBRA, such as transgenic animals and introduction of human tissues or cells, other than stem cells, into an animal.
 - iv. The IACUC should be familiar with the latest information concerning the [3rd Schedule of HBRA](#) and must not allow restricted research to proceed unless the PI has obtained MOH approval.
 - b. Each institution's Institutional Review Board (IRB) (referred to as the Research Ethics Committee in some other countries) protects the rights and welfare of human subjects involved in biomedical and behavioural research activities being conducted under its authority.
 - i. The IRB reviews, approves and monitors the ethical aspects of research projects that involve human subjects and human tissues/cells/data.
 - ii. The IACUC must ensure that the PI produces documentation of IRB approval/waiver, prior to granting IACUC approval to the protocol. PI must ensure that all human tissue and/or cells used in animals have been approved by the IRB.
- III.3.1.3 The onus is on the PI to justify and explain his/her proposed experiments to the satisfaction of the IACUC. Refer to [III.4](#).

III.3.1.4 If the IACUC does not have the scientific and technical expertise to evaluate all aspects of a protocol, it may bring in an outside expert consultant(s) to provide information.

- a. Such consultants may not vote on the protocol.

III.3.2 Procedures for protocol review

III.3.2.1 Institutions may develop their own meeting procedures, provided that the procedures are consistent and do not contradict the NACLAR Guidelines.

III.3.2.2 Full Committee Review

- a. Review of new protocols, and review of proposed significant changes to previously approved protocols should, with few exceptions (see below), receive Full Committee Review in a convened meeting of the IACUC.
- b. IACUCs may designate members to serve as primary and/or secondary reviewers.
 - i. Primary and secondary reviewers, usually chosen for their expertise or familiarity with a given topic, are responsible for an in-depth review of a protocol and for describing the protocol to the full IACUC and answering questions about the protocol during review by the IACUC.
 - ii. Primary and secondary reviewers may contact the PI prior to the meeting for clarifications, additional information, or in anticipation of questions the IACUC may raise.
- c. In some cases, the participation of the PI at the IACUC meeting may facilitate the review by addressing questions raised by the IACUC in a collegial exchange of information. However, the PI may not participate in the final IACUC deliberations and must not vote.

III.3.2.3 Designated Members Review (DMR)

- a. The IACUC may allow DMR protocols in lieu of a Full Committee Review. In this regard, the IACUC must develop a policy on what can be approved by DMR. For new protocols not covered by the policy, the DMR may occur only after all IACUC members have been provided with a list of the protocols to be reviewed and have an opportunity to call for a Full Committee Review. A DMR should be conducted by the AV, or the AV alternate, and at least one other member of the IACUC as assigned by the IACUC Chair.

- b. Examples of protocols that may be acceptable for DMR may include, but may not necessarily be limited to, those using small numbers of animals with procedures not expected to result in pain or distress, those using only non-survival surgery and those involving only euthanasia for tissue harvest. The IACUC is to determine whether DMR is appropriate in the circumstances, bearing in mind the general principle that Full Committee Review is still the preferred method.
 - i. Where a protocol is approved by DMR in lieu of a Full Committee Review, the IACUC must endorse the DMR approval.
 - ii. Where a protocol is not approved by all members of the DMR, it should be returned for Full Committee Review.

III.3.3 Categories of IACUC action on protocols

III.3.3.1 Following review of a protocol, an IACUC must take one of the following actions: (i) grant approval, (ii) require modifications before approval or (iii) withhold approval of the protocol. Alternatively, if major clarifications are required from the PI, the IACUC may defer or table a decision until a subsequent meeting.

III.3.3.2 The IACUC must notify the PI in writing of its decision to approve, withhold approval or require modifications to secure approval.

- a. If approval is withheld, the IACUC must provide the reasons for its decision and give the PI an opportunity to respond and request the IACUC reconsider its decision.
 - i. The IACUC must notify the PI in writing of its decision to defer the protocol until a subsequent meeting, to allow the PI to respond or make consequential plans accordingly.

III.3.3.3 Approval

- a. The IACUC must not grant its approval unless it is satisfied following the review that the following requirements are fulfilled:
 - i. any procedure involving the animal will be carried out in a manner that will avoid or minimise discomfort, pain or distress to the animal;
 - ii. the PI has considered alternatives to any procedure that may cause more than momentary or slight pain or distress to the animal and has provided a written and narrative description of the methods and sources used to determine that such alternatives are not available;
 - iii. the PI has provided written assurance that the project does not unnecessarily duplicate previous experiments;

- iv. the PI has provided a justification for the endpoints of the experiments to be carried out in the project;
 - v. the rationale for involving animals and the appropriateness of the species and the number of animals to be used justifies such use of the animals in the project;
 - vi. any procedure that may cause more than momentary or slight pain or distress to the animal — (i) will be carried out in consultation with the AV; (ii) will not include the use of paralytics without anaesthesia; and (iii) will be performed with appropriate sedatives, anaesthetics or analgesics, unless the withholding of such agents is justified for scientific reasons by the PI in writing and will be continued for only the necessary period of time;
 - vii. the animal will undergo euthanasia as soon as possible if it experiences severe or chronic pain or distress that cannot be relieved;
 - viii. the personnel who will conduct procedures on the animal are appropriately qualified and trained in such procedures and any trainee involved in the conduct of such procedures will be under appropriate supervision;
 - ix. in the case of any protocol that involves surgery, appropriate pre-operative and post-operative care for the animal will be provided in accordance with established veterinary practices and all survival surgery will be performed using aseptic procedures and aseptic techniques;
 - x. the animal will not be used in more than one experiment, unless the subsequent experiment is justified for scientific reasons by the PI in writing and such repeated use of the animal complies with the NACLAR Guidelines (refer to [II.11.6](#)); and
 - xi. the AV is consulted on the use of appropriate euthanasia on the animal.
- b. When the IACUC has determined that all review criteria (including but not limited to the above) have been adequately addressed by the PI, the IACUC may approve the protocol, thus providing the PI permission to perform the experiments or procedures as described.
- c. An IACUC-approved protocol may be subject to further review by the IO, who may reverse the protocol approval due to financial, policy, facility, or other institutional or administrative considerations. However, the IO may not approve a protocol if it has not been approved by the IACUC.

- d. Modifications required to secure approval
 - i. An IACUC may require modifications to the protocol before granting approval.
 - ii. If the IACUC determines that a protocol can be approved contingent upon receipt of a very specific modification (e.g. receipt of assurance that the procedure will be conducted in a fume hood), or clarification of a specific point, the IACUC may handle these modifications or clarifications as administrative details, that the IACUC Chair could approve.
 - iii. If a protocol is complex or involves untried or controversial procedures, the IACUC may wish to impose restrictions (e.g. approval for the use of a limited number of animals as a pilot study with a written report of interim results, or close monitoring by veterinary or other qualified personnel).
 - iv. If modifications require significant changes to the protocol, the PI must revise the protocol to reflect the modifications required by the IACUC.
 - v. If the protocol is missing substantive information necessary for the IACUC to make a judgement, or the IACUC requires extensive or multiple modifications, then the IACUC can vote to require that the protocol be revised and resubmitted.
- e. Anything less than full IACUC approval, via one of the accepted methods described above, is not adequate for initiation of animal activities. i.e. full IACUC approval must be received before initiation of any animal activities.
- f. IACUCs must not use terms such as “conditional approval,” “provisional approval”, “restricted approval”, “approved in principle” or “approved pending clarification” as this may be taken as approval to proceed with the protocol by the PI.

III.3.3.4 Withhold approval

- a. When the IACUC determines that a protocol has not adequately addressed the requirements of the NACLAR Guidelines, or other appropriate regulations and guidelines, the IACUC may withhold approval.
- b. As indicated above, a higher institutional authority may not overrule an IACUC decision to withhold approval of a protocol.

III.3.3.5 Defer or table review

- a. If the protocol requires clarification in order for the IACUC to make a judgement, and if IACUC members with appropriate expertise are not present, or the IACUC wishes to seek external consultation, or if there are other reasons preventing the IACUC from conducting its review, then the IACUC may wish to defer or table the review.
- b. Good communication between the IACUC and the PI can help to ensure that deferral or tabling is needed infrequently.

III.3.4 Review of amendments to approved protocols

- III.3.4.1 Changes/amendments to an IACUC-approved protocol must be reviewed and approved by the IACUC before they occur.
- III.3.4.2 The IACUC should develop a policy on the types of changes/amendments that are considered significant or minor, and how to review these changes to avoid ambiguity.
- III.3.4.3 Review of significant changes or amendments to approved protocols should be conducted by Full Committee Review.
- III.3.4.4 The following examples may guide the IACUC in its determination of whether a change is significant or minor. Significant changes include:
 - a. change in objectives of the study,
 - b. increase in degree of invasiveness of a procedure or discomfort to an animal,
 - c. change in species,
 - d. change in anaesthetic agent(s) or in the use or withholding of analgesics,
 - e. change in methods of euthanasia,
 - f. change in duration, frequency or number of procedures performed on an animal.
 - g. proposals to switch from non-survival to survival surgery, and
 - h. increase in number of animals requested.

III.3.5 Frequency of review of approved protocols

- III.3.5.1 The IACUC must conduct a review of activities under approved protocols at least once annually.
- III.3.5.2 Investigators must annually report to the IACUC on the status of the protocol, verify that completed activities were conducted in accordance with the approved protocol, describe any departures from the approved protocol, and provide information about activities projected.
- III.3.5.3 A completely new protocol submission and review, in other words “*de-novo*” review, must occur within 4 years of the initial protocol approval with the possibility of a 1-year extension if justified and approved by the IACUC.

III.4 **Protocol Review Criteria**

III.4.1 Alternatives – replacement, reduction and refinement (3Rs)

- III.4.1.1 The IACUC must ensure that PIs have appropriately considered alternatives (including non-animal methods) and refinements to procedures that can cause more than slight or momentary pain or distress in animals, consistent with sound research or instructional design. To this end, the PI must provide the IACUC with a description of the methods and sources used to determine that such alternatives are not available.
- III.4.1.2 The IACUC should refer to refer to [Section II: Guiding Principles](#).
 - a. Replacement – Refer to [II.4.2](#).
 - b. Reduction – Refer to [II.4.3](#).
 - c. Refinement – Refer to [II.4.4](#).
- III.4.1.3 Of the 3Rs, refinement of techniques to reduce or eliminate unnecessary pain and distress in study animals is the most commonly practised (see Russell and Burch, 1959 in [Appendix I: Section III: The IACUC](#)). PIs must consider alternatives to painful procedures, and to avoid or minimise distress and pain, consistent with sound scientific practice and the goals of the research. This requires an understanding of the potential of pain or distress in the animals.
 - a. Some refinement opportunities may include:
 - i. pain-relieving drugs,
 - ii. non-pharmacologic techniques for pain relief,

- iii. new diagnostic and therapeutic techniques,
 - a) New diagnostic and therapeutic techniques may have the capability to dramatically reduce the invasiveness of data collection.
 - b) Examples include use of imaging equipment to replace invasive procedures and allow chronological data collection on the same animal; blood and tissue sampling techniques that allow for easier collection; and the utilisation of smaller sample volumes/sizes.
- iv. behavioural management/environmental enrichment programmes, and
- v. establishment of humane endpoints.
 - a) The earliest possible humane study endpoint consistent with the research design must be used. Refer to [II.11.5](#).
 - b) For any study that defines death of the experimental animal as the endpoint, the PI must ensure, and the IACUC must be convinced, that there is no earlier point when the necessary data can be obtained and the animal could be euthanised. Refer to [II.11.5.3](#) and [III.4.4.4](#).

III.4.2 Minimising pain and distress

- III.4.2.1 It is the responsibility of the IACUC to critically evaluate all protocols for the potential to cause pain and/or distress, and assess the steps that are to be taken to enhance animal well-being. The IACUC must ensure that pain and distress are minimised in laboratory animals.
- III.4.2.2 The IACUC must ensure the protocol addresses:
 - a. appropriate sedation, analgesia and anaesthesia;
 - b. non-pharmacological methods to reduce pain and distress, such as special housing, dietary and other environmental enrichment, adjustments and careful supportive care; and
 - c. details of peri-procedural care.
- III.4.2.3 The IACUC's deliberations regarding the management of potential pain and distress in a protocol should be documented.
- III.4.2.4 Assessing Pain and Distress - Refer to [Appendix V: Signs of Animal Pain and Distress](#).

- a. The IACUC must ensure that animal care staff and researchers receive adequate training on how to recognise clinical signs of pain and distress.
- b. The IACUC should ensure that there is a mechanism in place for prompt reporting of animals in pain or distress to the AV.

III.4.3 Euthanasia - Refer to [II.11.9](#)

III.4.3.1 The IACUC must review and approve methods of euthanasia based on the following:

- i. minimum pain, distress, anxiety or apprehension;
- ii. minimum delay until unconsciousness;
- iii. reliability and irreversibility;
- iv. safety of personnel;
- v. emotional effect on personnel;
- vi. compatibility with protocol requirement and purpose, including subsequent use of tissue;
- vii. compatibility with species, age and health status;
- viii. drug availability and human abuse potential; and
- ix. consistency with the prevailing AVMA Guidelines for the Euthanasia of Animals (see [AVMA Panel on Euthanasia](#) in [Appendix I: Section II: Euthanasia](#)).

III.4.4 Humane endpoints - Refer to [II.11.5.2](#) and [CCAC](#) in [Appendix I: Section II: General](#)

III.4.4.1 Criteria used to end experimental studies earlier than the planned experimental endpoint, in order to avoid or terminate unrelieved pain and/or distress, are referred to as humane endpoints.

III.4.4.2 Animals used in research and testing may experience pain from induced disease, procedures, or toxicity. More than momentary or slight pain or distress should be alleviated with appropriate sedation, analgesia, anaesthesia, or other humane interventions.

- a. However, research and testing studies sometimes involve pain that cannot be relieved with such agents because they would interfere with the scientific objectives of the study.

- i. The IACUC must determine that any discomfort to animals will be limited to that which is unavoidable for the conduct of scientifically valuable research, and that unrelieved pain and distress will only continue for the minimum duration necessary to accomplish the scientific objectives.
- b. Animals that would otherwise suffer severe or chronic pain and distress that cannot be relieved should be euthanised at the end of the procedure, or if appropriate, during the procedure.

III.4.4.3 Developing Humane Endpoints

- a. An important feature of humane endpoints is that they should ensure that study objectives will still be met even though the study may need to be ended at an earlier point. Ideally, humane endpoints are sought such that they can be used to end studies before the onset of pain and distress. Animals having reached the humane endpoints should be treated or removed from the study, as outlined in the approved protocol.
- b. Humane endpoints must be used to determine when animals will be removed from the study, treated, or euthanised. There must be clear directions concerning who can make the decision to euthanise or treat animals in consultation with the AV, including procedures to follow if a situation arises on weekends, holidays, or in the absence of the responsible Investigator. The AV has the final say in a disagreement regarding euthanasia or treatment.
- c. The development and use of humane endpoints can reduce the severity and duration of unrelieved pain and distress. Humane endpoints must be defined in the protocol and approved by the IACUC.
- d. Moribund condition as an endpoint:
 - i. Moribund has been defined as “in the state of dying,” or “at the point of death.”
 - a) Moribund is not considered to be an appropriate humane endpoint. Euthanasia of animals prior to the moribund state can prevent further pain and distress.
- e. Animals should be observed at intervals frequent enough to detect defined humane endpoints so that appropriate interventions can be taken.
- f. Various clinical signs that may be used to determine humane endpoints may include one or more of the following (Refer to NEAC in [Appendix I: Section III: The IACUC](#) for sample animal score sheets, and [Appendix V: Signs of Animal Pain and Distress](#)):

- i. impaired ambulation that prevents animals from reaching food or water,
 - ii. excessive weight loss and emaciation (for example 20% weight loss compared to age matched controls, accounting for tumour burdens or ascites),
 - iii. lack of physical or mental alertness,
 - iv. difficult laboured breathing,
 - v. inability to remain upright, or
 - vi. poor body condition score.
- g. Other scoring methods or use of biomarkers, may be considered.
- h. When increased morbidity or mortality is expected, then animals should be observed more often (dependent on expected timing), and at least twice daily observation is recommended.
- i. Animals not likely to survive until the next scheduled observation should be euthanised.
 - ii. In situations where animals are often found dead, more frequent observation of remaining animals should be implemented and the criteria for humane endpoints should be reassessed.

III.4.4.4 Death as an endpoint

- a. Refer to [II.11.5.3](#) and [CCAC](#) in [Appendix I: Section II: General](#).
- b. Since it provides an objective and unequivocal data point, death historically may have been used as an endpoint in cancer, infectious disease and other animal studies, especially for regulatory purposes (e.g. drug safety/efficacy studies).
- c. Increased public interest and regulation have led to a re-evaluation of this practice. Much of the concern arose from the use of traditional LD 50 tests for chemicals and drugs to determine acute toxicity. However, in most instances regulatory testing requirements for acute toxicity now allow for animals that are exhibiting clinical signs of severe pain and distress to be euthanised rather than die spontaneously.
- d. Euthanasia also provides tissues or samples more appropriate for subsequent study and alleviates suffering of the animal.

- e. Hence euthanasia must be considered, rather than death, for both scientific and ethical reasons.
- f. Endpoints other than death must be considered and used whenever the research objective can be attained with non-lethal endpoints.

III.4.5 Personnel qualifications

- III.4.5.1 The IACUC must assess whether personnel conducting procedures are appropriately experienced or trained in those procedures.
 - a. A mechanism should be in place to ensure that new personnel are trained.
 - b. Evaluation of personnel qualifications and training are an essential component of the review of animal use protocols to ensure the humane care and use of laboratory animals.
 - c. The challenge to IACUCs is to perform this evaluation in an efficient, consistent and uniform manner. Refer to [Section IV: Training](#) for more details.

III.4.6 The use of hazardous materials

- III.4.6.1 The IACUC must pay particular attention to protocols employing potentially hazardous materials, including:
 - a. radioactive substances,
 - b. infectious microorganisms,
 - c. biological toxins,
 - d. hazardous chemicals,
 - e. nanomaterials, and
 - f. recombinant DNA.
- III.4.6.2 Hazardous materials have the potential for causing harm to research animals, researchers and the personnel caring for and working with them. The IACUC must engage the IBC, Chemical Safety, Radiation Safety, or appropriate institutional entity as appropriate, to review the use of these hazardous materials before approval by the IACUC.
- III.4.6.3 Refer to [Section V: Occupational Health and Safety in Animal Care and Use](#).

III.4.7 Use of pharmaceutical-grade chemicals and other substances

- III.4.7.1 A pharmaceutical-grade substance is defined as any active or inactive drug, biologic or reagent, for which a chemical purity standard has been established by a recognised national or regional pharmacopeia (e.g. the U.S. Pharmacopeia (USP), British Pharmacopeia (BP), National Formulary (NF), European Pharmacopoeia (EP), Japanese Pharmacopeia (JP) etc.).
- These standards are used by manufacturers under Good Manufacturing Practice controls to help ensure the products are of the appropriate chemical purity and quality, in the appropriate solution or compound, to ensure stability, safety, and efficacy.
 - The use of pharmaceutical-grade chemicals and other substance ensures that toxic or unwanted side effects are not introduced into studies conducted with experimental animals.
- III.4.7.2 Whenever possible, pharmaceutical-grade substances must be used for the clinical treatment of animals; and to prevent, reduce or eliminate animal pain or distress (e.g. anaesthetics, analgesics, antibiotics, euthanasia agents), even in non-survival procedures.
- Non-pharmaceutical grade substances should only be used in clinical treatments after specific review and approval by the IACUC for reasons such as scientific necessity or non-availability of an acceptable veterinary or human pharmaceutical-grade product.
- III.4.7.3 When a pharmaceutical-grade substance is formulated with any excipient, such as vehicle, solvent or diluent, the excipient should be of pharmaceutical-grade.
- Commonly used solvents or vehicles such as normal saline, phosphate buffered saline (PBS) and dimethyl sulfoxide (DMSO) are available in pharmaceutical-grade.
 - Pharmaceutical-grade substances adulterated by non-pharmaceutical-grade excipients will render the preparation non-pharmaceutical-grade.
- III.4.7.4 For research purposes, i.e. when the compounds are used to accomplish the scientific aims of the study, pharmaceutical-grade substances must be used if available, and suitable.

- III.4.7.5 When non-pharmaceutical-grade substances, or pharmaceutical-grade substances adulterated with non-pharmaceutical-grade excipients are used, consideration should be given to the grade, purity, sterility, pH, osmolality, stability, site and route of administration, formulation, compatibility, toxicity, pyrogenicity, side effects and pharmacokinetics of the preparations to be administered.
- III.4.7.6 If the use of non-pharmaceutical-grade substances is essential for the conduct of science, the goal of the IACUC is to consider the health and well-being of the animals while aiding the PI in minimising potentially confounding experimental variables and maximising reproducibility of the research.
- III.4.7.7 Situations when the use of non-pharmaceutical-grade substances are acceptable include:
- a. the pharmaceutical-grade compound is not available in the appropriate concentration or formulation or the appropriate vehicle control is unavailable in pharmaceutical-grade; and
 - b. the chemical properties of the compound and the route of administration are appropriate for the study (e.g. the purity, grade, stability in and out of solution, solution vehicle properties, pH, osmolality, and compatibility of the solvent and other components of the final preparation).
 - i. Best practices for administration of substances should be followed when injecting non-pharmaceutical grade compounds into animals.
- III.4.7.8 If a novel compound is being tested, a pilot study using a minimum number of animals should be considered to determine the correct dosage and potential for any induced adverse effects. This, in turn, will help determine humane end point criteria specific to the project.

III.4.8 GM animals – Refer to [II.6.1.4](#)

- III.4.8.1 If a protocol proposes the use of a spontaneous or induced mutant model and the mutant animal can be purchased from a resource or commercial colony, review of this protocol is similar to review of any other protocol.
- III.4.8.2 If a protocol uses an induced mutant model and only breeders are available from the source, review of this protocol is similar to review of any other breeding colony.

III.4.9 Food and fluid regulation/restriction

- III.4.9.1 Regulation, or restriction, of food or fluid intake may be required for the conduct of some physiological, neuroscience, and behavioural research protocols.
- III.4.9.2 Regulation entails scheduled access to food or fluid sources, so an animal consumes as much as desired at regular intervals.
- III.4.9.3 Restriction is when the total volume of food or fluid consumed is strictly monitored and controlled.
- III.4.9.4 The PI must describe how the fluid balance and/or body weight will be monitored, recorded and maintained consistent with the limits approved by the IACUC.
 - a. This should include the frequency of monitoring and criteria for removal of the animal from food and/or fluid regulation or restriction.
- III.4.9.5 Food and fluid restriction should be limited to the shortest period possible for the conduct of the research.
- III.4.9.6 Food and fluid restriction must not compromise the health of the animals.
- III.4.9.7 If an alternative to food and fluid restriction is available (e.g. food rewards for positive reinforcement in behavioural research), it should be used with priority over food and fluid restriction.

III.4.10 Physical restraint – Refer to [II.11.8](#)

- III.4.10.1 Physical restraint is the use of manual or mechanical means to limit some or all of an animal's normal movement for the purpose of examination, collection of samples, drug administration, therapy, or experimental manipulation.
- III.4.10.2 The PI must describe methods of restraint, time of restraint, length of restraint, and any training or conditioning planned to help the animals cope with restraint.
 - a. If an animal does not adapt to restraint despite habituation, it should be removed from the study or other appropriate accommodations made to accomplish the purpose of the study.

III.4.11 Tumour studies

- III.4.11.1 Animals used to study tumour biology, to develop new cancer therapies, and to evaluate the carcinogenic potential of substances may experience pain and distress.
- III.4.11.2 Frequent and appropriate monitoring of animals during tumour development is necessary to allow for appropriate intervention before significant deterioration or death.
- III.4.11.3 Effective monitoring systems and endpoints should include limits on tumour size and severity of tumour-associated disease – for example, interference with locomotion or feeding/drinking or an ulcerated tumour.
- III.4.11.4 Altered physiologic, biochemical, and other biomarkers may be potentially more objective and reproducible endpoints than clinical signs for such studies.
- III.4.11.5 The site for induction of tumours should be chosen carefully. Subcutaneous, intradermal and flank sites should be chosen wherever possible. Prior to the use of footpad, brain and eye sites, specific scientific justification should be provided as to the lack of a comparable alternative.
- III.4.11.6 With ascitic tumours, including hybridomas, the volume of ascitic fluid should not cause gross abdominal distension.
- III.4.11.7 In tumour therapy experiments, the endpoints chosen should be as early as possible and be compatible with reliable assessment of the therapy.
- III.4.11.8 Bearing in mind that tumour mass increases over time, changes in body weight or body condition that takes into account the weight of the tumour and compared to age-matched controls, should be monitored closely.

- III.4.11.9 Death from the tumour should not be chosen as an experimental end-point. Refer to [II.11.5.3](#), [III.4.4.4](#) and CCAC in [Appendix I: Section II: General](#).

III.4.12 Infectious disease studies

- III.4.12.1 Animals with induced infections may experience significant pain and/or distress during progression of the disease.
- III.4.12.2 Early physiologic and biochemical changes during infection have been found to be useful humane endpoints rather than death or disease. Specific decreases in body temperature have been found to be effective early predictors of eventual death for some infections in rodents. Refer to [II.11.5.3](#) and [III.4.4.4](#).

III.4.13 Vaccine studies

- III.4.13.1 Vaccine potency testing typically involves challenging immunised animals with infectious agents. While such testing has commonly used death as the endpoint indicative of insufficient protection, some regulatory authorities now allow early euthanasia of affected animals. Alternatives to death as an endpoint should be implemented, when permitted. Refer to [II.11.5.3](#) and [III.4.4.4](#).

III.4.14 Toxicity testing

- III.4.14.1 Investigation into the safety of agents intended for use in humans, animals, the household or the environment, or investigation of naturally occurring toxins, should be performed by persons with appropriate training.
- III.4.14.2 If suitable non-animal tests are available, they must be used. In particular, in vitro methods should be used as an initial screening test, whenever possible.
- III.4.14.3 Animals in toxicology studies in pain or showing signs of severe and enduring distress should be euthanised.
- III.4.14.4 Humane endpoints must be established and used for toxicology studies to minimise pain and distress.
- III.4.14.5 The end-point of such experiments must be as early as is compatible with reliable assessment of toxicity and must minimise the extent of any pain and distress.
- III.4.14.6 Animal testing done based on internationally recognized protocols and quality assurance systems like Good Laboratory Practices (GLP), which facilitate mutual acceptance of data, can significantly reduce animal test requirements.

III.4.15 Antibody production

III.4.15.1 Antibodies are important tools for research. Depending on research needs, antibodies may be produced by polyclonal or monoclonal technique. Each technique requires that specific issues be addressed in the animal protocols.

III.4.15.2 There are commercial in vitro sources of antibodies made to order. These should be considered, when possible, as an alternative to in-house production using animals.

III.4.15.3 Adjuvants

- a. To increase the immune response, the immunogen may be combined with an adjuvant.
- b. The choice of the appropriate adjuvant is important from both the aspect of the end result (high antibody response) and the welfare of the immunised animal. Many adjuvants have the capacity to cause inflammation, tissue necrosis and pain in animals. A major charge to PIs is to minimise animal use and discomfort.
- c. Incomplete Freund's adjuvant (IFA) is a water/oil emulsion containing immunogen, paraffin oil and an emulsifying agent. Addition of killed mycobacteria to the oil phase (Complete Freund's adjuvant, CFA) enhances the immune response.
 - i. Multiple exposures to CFA will cause severe hypersensitivity reactions. Abscesses, granulomas and tissue sloughs may occur at injection sites.
 - ii. If CFA has been used in the initial injection, IFA must be used for booster antigen administrations, due to the severity of the secondary immune response to mycobacterium in CFA.
 - iii. Adjuvants other than CFA are available as alternatives and must be considered.
- d. Route of Injection
 - i. The range of recommendations for routes and sites of administration of antigen-adjuvant preparations, volumes per site and number of sites per animal for different species vary in the literature and institutional guidelines.

- ii. Particularly with the use of CFA, it is important to note that the severity of painful inflammatory reactions can be minimised by injection of a small volume of inoculum at each site, i.e. multiple injection sites of a smaller volume should be used. See [OACU](#) in [Appendix I: Section III: The IACUC](#).
- e. Injection sites must be sufficiently separated to prohibit coalescing of the inflammatory lesions.
 - i. Using multiple sites for immunisation also provides more loci for antigen presentation and the involvement of more lymph nodes.
- f. Intradermal and subcutaneous routes are commonly used to take advantage of antigen-processing dendritic cells present within the dermis.
 - i. Hair should be clipped from intradermal and subcutaneous injection sites.
 - ii. The site should be aseptically prepared with an iodine or chlorhexidine scrub followed by alcohol or another appropriate antiseptic.
- g. Footpad injections:
 - i. Are not permitted for antibody production or other purposes, except in rodents when scientific justification is provided and approved by the IACUC. Hock injections are considered less traumatic.
 - a) Only one hind foot/hock should be injected and the rodents should be housed on soft bedding to reduce discomfort.
 - b) Suggested maximum injection volumes can range from 0.01 to 0.05 for mice and to 0.10 ml for rats.
 - c) The need for footpad injections must be critically evaluated by the IACUC before approval.
- h. Sometimes direct inoculation into lymph nodes, such as the popliteal lymph node, is used. With practice these nodes often can be palpated and the injection performed percutaneously.

III.4.16 Breeding colonies

III.4.16.1 PIs proposing to maintain breeding colonies should include the following information in their protocol to assist the IACUC in its deliberations:

- a. the breeding system;
- b. colony management practices;
- c. the estimated number of animals including the:
 - i. number of breeders needed to obtain needed offspring,
 - ii. number of young that will not be used in experiments because they are of the wrong genotype or gender; and
 - iii. number of animals that will be subject to experimental manipulations.

III.4.16.2 If a study requires fertilised one-cell eggs, embryos or fetuses, the protocol should consider the number of eggs, embryos or fetuses that are required for proposed studies to estimate the appropriate number of animals for the study.

- a. The estimated number of experimental animals may be limited to the number of female animals that are mated and euthanised or surgically manipulated to collect the required eggs, embryos or fetuses.
- b. The males can be listed as breeders if they are not subject to any experimental manipulation.

III.4.16.3 Determining the estimated number of animals for breeding can be challenging to the PI and the IACUC in the absence of IACUC-developed guidelines.

- a. One option is for the IACUC to request estimated animal numbers as follows:

	Estimated number
Animals, including unweaned animals, to be used for experimental manipulations	
Breeders held but not subjected to experimental manipulations	
Animals to be euthanised and not used for experimental manipulations (e.g. animals of inappropriate genotype, wrong sex, etc.)	

III.4.16.4 PIs may submit, and IACUCs may approve, a separate protocol for breeding. Measures to prevent genetic drift, where appropriate, should be in place.

- a. Animals under a breeding protocol must not be used for any experimental manipulations before they are transferred to an approved experimental protocol.
- i. This option is particularly beneficial for PIs having multiple experimental protocols where animals generated from the breeding protocol are transferred to these various protocols for experimental manipulations.
 - ii. Records must be maintained, by the PI, of the number of animals transferred from a breeding protocol to experimental protocols.
 - a) The animals transferred to an experimental protocol should be reported as used under the experimental protocol, not the breeding protocol. Refer to [II.9.3.1.b. iv-vii](#).
- b. Other than serving the PI's own protocols, animals from the breeding protocols may serve other PIs' protocols within the institution or at other institutions.
- i. This practice may reduce the number of animals bred of the same species and strain, and decrease the number of animals wasted because of the wrong size, sex, etc.

- ii. The IACUC should determine if the practice is beneficial and provide a mechanism for the method of animal transfer from breeding to experimental protocols.
- c. The PI should keep an accurate record of the number of animals produced, weaned and the number of breeders used.
- d. The IACUC should develop a method of monitoring animal breeding activities and records.

III.4.16.5 In summary, the IACUC's role for oversight regarding breeding colonies includes ensuring that the need for a breeding colony has been established based on scientific or animal welfare concerns, that the procedures used in the breeding colony are evaluated and approved by the IACUC on a regular basis (e.g. as part of the annual programme review), and that there is a mechanism for tracking animal use and ensuring that production is kept to the minimum required.

III.4.17 Field studies

III.4.17.1 Field studies often pose unique challenges to the PI and the IACUC because of the nature of field research. For example, field sites are often at a distance and may be remote, making it impractical for IACUC inspections.

- a. Observation of animals, without any type of interaction with them and/or manipulation of their environment, do not require IACUC approval.

III.4.17.2 Other difficulties relate to the nature of the research and the populations to be studied, which may be unfamiliar to the IACUC.

III.4.17.3 Professional field biologists in organisations devoted to the study of fish, amphibians, reptiles, birds, and mammals have prepared guidelines for field work with these populations. These guidelines form a useful reference and can assist the PI in planning, and the IACUC in reviewing, field research using vertebrate animals (see Fair et al., 2010; [HACC, 2004](#); [Sikes et al., 2011](#) in [Appendix I: Section III: The IACUC](#)).

III.4.17.4 Legislation protecting animal and wildlife populations include:

- a. the [Animals and Birds Act \(Cap. 7\)](#), the [Wildlife Act \(Cap. 351\)](#) and the [Endangered Species \(Import and Export\) Act \(Cap. 92A\)](#) (see [Appendix I: Reference Materials: Legislations](#)). Refer to [II.6.2](#) and [II.6.3](#).

III.4.17.5 The PI must provide evidence to the IACUC that all necessary licences and permits have been or will be obtained before the research begins.

III.4.17.6 The PI should address the following relevant items:

- a. species selection,
- b. site selection, and
- c. methodologies employed.

III.4.17.7 Site Selection

- a. The selection of the study site for the research should maximise the opportunity for data collection and minimise the disruption caused by the research.
- b. The site selection should also take into consideration other activities in the area, such as agricultural use, tourism or land development, which may interfere with the research protocol.
- c. Permission to utilise the site may be necessary and the PI must be able to assure the IACUC that necessary permits or permissions have, or will be obtained, before the research begins.

III.4.17.8 Methods Employed

- a. The potential short- and long-term effects of procedures on individual animals should be evaluated.
- b. If animals are to be captured, the capture methods used and the numbers to be captured must be detailed in the protocol.
 - i. A description of measures taken to prevent injuries and alleviate potential distress, the possible impact of capture on subsequent behaviour and survival of the animals, and capture of non-target species must be described.
 - ii. If animals are to be identified individually, the PI must indicate whether they will be identified by natural markings or will be artificially marked.
- c. If the animals are to be artificially marked, there must be a description of marking methods and potential trauma (e.g. paint markings may increase visibility to predators).
- d. If field experimental procedures are used, any potential pain or distress to an animal must be assessed and evaluated in the context of the potential value of the data to be obtained.

- e. Techniques for remotely recording behavioural or physiological data in the field are valuable and often minimally invasive.
- f. When possible, the least invasive procedures should be chosen (e.g. use of hormone assays of urine or faeces rather than blood samples).
- g. When it is necessary to take measurements or tissue samples from animals, the IACUC should evaluate the degree of invasiveness of the procedure and potential problems associated with return of the animal to the field. For example, animals should be released in a condition that enables them to avoid predators, seek shelter, and survive inclement weather.
- h. Any surgery must be done using aseptic techniques.
- i. The use and choice of anaesthesia will be affected by field conditions because some agents are difficult to transport or use in field conditions.
- j. Anaesthetics that do not clear from the system quickly may require holding the animal longer as they may compromise the animal's ability to survive when released.
- k. The potential for human consumption of game species administered anaesthetics, antibiotics, other drugs or compounds must be considered.
- l. Procedures involving sites or environmental manipulation must be described and adequately justified by the PI.

III.4.17.9 Field study issues may be difficult to address definitively, but their consideration will help the IACUC judge the potential impact and value of the protocol proposed, and will assist the PI in obtaining maximum information from the study with minimum negative impact on the animals studied or their environment.

III.4.18 Instructional use of animals

- III.4.18.1 All instructional use of animals, must be reviewed by the IACUC.
- III.4.18.2 Instructional use includes, but is not limited to, animal handling, training and surgical workshops.
- III.4.18.3 It may be appropriate for students to participate in the conduct of experiments involving research animals for the purpose of education.
- III.4.18.4 Instructional protocols must clearly identify the learning objectives and justify the value of animal use as part of the course.

- III.4.18.5 Adequate supervision and training are especially important as the techniques learned by students may be carried into subsequent research careers or even human patient care.
- III.4.18.6 When students work in a PI's laboratory, the PI must assure the IACUC that the students receive appropriate supervision and training in animal care and use.
- III.4.18.7 For all protocols, including those in which animals are euthanised to obtain tissues (e.g. in the teaching of anatomy or tissue harvest for in vitro procedures), the procedures and method of euthanasia, if any, must be reviewed by the IACUC.
- III.4.18.8 The number of animals used must be the minimum necessary to accomplish the objectives of the proposed educational activity.

III.4.19 Surgery

- III.4.19.1 Refer to [II.11.10](#).

III.4.19.2 Definitions

- a. Major surgery: Penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic functions.
- b. Minor surgery: Does not expose a body cavity and causes little or no physical impairment.
- c. Survival surgery: The animal awakes from surgical anaesthesia.
- d. Non-survival surgery: The animal is euthanised before recovery from anaesthesia.

III.4.19.3 Some of the aspects of a surgical procedure that the IACUC reviews are:

- a. details of the procedure (e.g. the actual procedure itself, pre- and post-operative care, aseptic technique, sequence of multiple procedures);
- b. appropriateness of the species for the procedure proposed;
- c. qualifications of the personnel performing the surgical procedures;
- d. species-specific and procedure-specific facility requirements; and
- e. occupational health and safety issues.

- III.4.19.4 Surgical procedures proposed may be experimental and require ongoing review by the IACUC as the procedure is developed.
- III.4.19.5 Major survival surgery in non-rodents requires dedicated surgical facilities.
- III.4.19.6 For most survival surgeries in rodents, and other small species (e.g. zebrafish, medaka, *Xenopus* etc.), a dedicated space in an animal procedure room or laboratory will suffice. The dedicated space should only be used for surgery and related activities and managed to minimise contamination from other activities in the room.
- III.4.19.7 The IACUC must assess the availability of the necessary facilities during the protocol review process.

III.4.20 Multiple survival surgery (MSS)

- III.4.20.1 Animals may not be used in MSS unless:
 - a. there is a scientific justification (e.g. related components of the same study) provided by the PI, and approved by the IACUC;
 - b. the MSS is required for a routine veterinary procedure or to protect the health and well-being of the animal, as determined by the AV;
 - c. under other special circumstances which have been approved by the IACUC; and
 - d. the provisions of [II.11.6](#) (repeated use of animals in experiments) are not breached.
- III.4.20.2 Subsequent to approval of MSS, the IACUC should ensure that there is sufficient ongoing oversight of the project.

III.4.21 Use of paralytic agents – Refer to [II.11.12](#)

III.4.22 Patient monitoring

- III.4.22.1 The sophistication of patient monitoring required varies with the species and the procedure, but during protocol review, the IACUC should expect evidence of the following:
 - a. a pre-surgical assessment;
 - b. adequate monitoring of depth of anaesthesia and animal homeostasis during the surgical procedure;
 - c. support such as fluid supplementation, external heat and ventilation;

- d. monitoring and support during anaesthetic recovery; and
- e. post-surgical monitoring details, (e.g. what will be done and how often, who will be responsible, and the name and phone number of the individual to contact in the case of post-surgical complications).

III.4.23 Record-keeping

- III.4.23.1 Record-keeping is an essential component of peri-operative care.
- III.4.23.2 Post-operative records, at a minimum, should reflect that the animal was observed until it was extubated and had achieved sternal recumbency.
- III.4.23.3 Records must include administration of analgesics and antibiotics (if required), other medications, basic vital signs, monitoring for infection, wound care, and other medical observations.
- III.4.23.4 For all surgical procedures on non-rodent mammals, an intra-operative anaesthetic monitoring record must be kept and included with the animal's records.

III.5 Post-Approval Monitoring (PAM)

III.5.1 PAM is a part of IACUC oversight responsibilities for on-going assessment of protocol conduct and compliance. PAM also provides opportunities to refine procedures.

III.5.2 The methods and mechanisms of PAM may vary depending on the animal care and use programme. The IACUC should establish the methods and mechanisms best suited to the programme.

III.5.3 The effective monitoring of animal use specific to a protocol may include direct assessment, review of records, and discussion of the issues such as proper methods and techniques (e.g. euthanasia, transporting animals to or from the animal facility to procedure rooms outside the animal facility; aseptic surgical technique; storage of drugs and controlled substances, etc.).

III.5.3.1 Records to be reviewed include:

- a. Animal monitoring records (frequency of monitoring and parameters monitored for tumour growth, humane endpoint criteria food and/or fluid regulation, etc.).
- b. Anaesthesia records, surgery and post-surgery records; analgesia; controlled drugs; breeding records etc.
- c. Records of monitoring for procedures with special considerations (e.g. novel procedures, pain studies; studies approved with exemptions from the NACLAR Guidelines or IACUC policies, etc.).

III.5.4 PAM is more likely to succeed when an educational partnership with the research team is emphasised.

III.5.5 The IACUC and AV must have appropriate access at all times to animals, animal records, and areas of animal care and use, including procedure rooms outside of the animal facility.

III.5.5.1. Video may be considered as an accepted alternative to direct access in exceptional cases.

III.5.6 While oversight responsibility lies in the hands of the IACUC, other personnel may be authorised by the IACUC to conduct PAM on its behalf and to report non-compliance to the IACUC.

III.5.7 PAM should consist of at least the following:

III.5.7.1 Annual review of activities conducted under each protocol, including:

- a. the number of animals used in each pain/ distress category;

- b. unexpected deaths;
- c. other adverse events, unanticipated outcomes or unpredicted phenotypes affecting animal well-being;
- d. any changes or refinements in the protocol; and
- e. progress over the previous year.

III.5.7.2 Monitoring and reporting of adverse events and unexpected outcomes.

- a. Adverse events or unexpected outcomes may negatively affect animal well-being and/or introduce a new variable to a study.
- b. Adverse events may be a result of deficiency in procedures, inadequate monitoring, faults in study design, or lack of training. However, unexpected outcomes or unpredicted phenotypes may also occur from novel procedures, new model development, or new GM animals.
- c. The PI and research team members must monitor and report adverse events, unexpected outcomes or unpredicted phenotypes to the AV and the IACUC.
- d. When applicable, the protocol should be amended, to establish additional monitoring parameters and criteria for humane endpoints.
- e. More frequent monitoring may be required for novel procedures, new model development, or new GM animals.

III.5.7.3 Animal acquisition and tracking:

- a. The IACUC must establish mechanisms to monitor and document the number of animals acquired and used in approved activities.
- b. PIs with breeding colonies must maintain accurate records and report all animals annually in the appropriate categories as provided in the Guidelines. Refer to [II.9.3.1.b.iv-viii](#) and [III.4.16.3](#).

III.5.7.4 Annual inspection

- a. During annual facility inspections, IACUC members should note the use of animals and may verify that the observed procedures are consistent with the approved protocol.

III.5.8 Additional PAM methods:

III.5.8.1 Veterinary staff and/or the IACUC may observe animals or procedures:

- a. for selected protocols;
- b. new methods or procedures, new models, or pilot studies; and
- c. following a report of non-compliance or unexpected outcomes.

III.5.8.2 Observations, records, and reports by animal care, veterinary and IACUC members or researchers.

- a. Veterinary staff are in a position, through periodic visits to the animal facility and animal activity areas, to observe and evaluate animal well-being and decide whether the animal activities are being conducted in accordance with the conditions described or referenced in the approved protocol.
- b. Research, veterinary, and husbandry staff should be aware of SOPs, IACUC policies, and information about what has been approved in the protocol. Appropriate information should be made accessible to research, veterinary and husbandry staff.
- c. All staff must be free to report perceived deviations to the IACUC, which must then consider such concerns. Refer to [III.6](#).

III.5.8.3 Compliance Staff:

- a. should be knowledgeable with local guidelines/regulations, standards of animal care and use, and safety requirement within the facility;
- b. should have laboratory animal training and experience;
- c. should be authorised to conduct announced or unannounced laboratory inspections on behalf of the IACUC;
- d. may periodically survey individual laboratories and/or research teams (whether conducting procedures within or outside of the animal facility) to ensure that procedures, monitoring, and records are consistent with approved protocols;
- e. may visit procedure areas;
- f. may meet with PIs and/or research staff to review procedures, records, or concerns;
- g. should provide written reports of their activities to the IACUC, including any deviations or instances of non-compliance; and

- h. may be full or part-time compliance staff to monitor procedures on behalf of the IACUC.

III.5.8.4 External regulatory inspections and assessments may be considered additional forms of PAM.

III.5.8.5 Conclusion:

- a. It is the responsibility of PIs to understand the procedures and the details approved in their protocol, and to ensure that there are no deviations.
- b. The IACUC can help establish a climate of compliance to ensure that animal use conforms to ethical and welfare standards, laws, regulations, rules, and policies.

III.6 Animal Welfare Concerns

III.6.1 General

- III.6.1.1 To help ensure that research animals receive humane care and use or treatment in accordance with the highest ethical standards, laws, regulations and policies governing animal research, the IACUC must review and, if warranted, address any animal welfare concerns raised by the public or institutional employees.
- III.6.1.2 Procedures must be established to ensure that concerns are communicated to the IACUC.
- III.6.1.3 The IACUC must review each concern in a timely and systematic manner and, when necessary, take prompt, appropriate corrective actions.

III.6.2 Policy for reporting animal care and use concerns

- III.6.2.1 The IACUC must develop and implement policies and procedures to ensure that animal care and use concerns are brought to the IACUC's attention.
- III.6.2.2 Some of the elements that should be included in these policies and procedures are described in [III.6.2.3-4](#) and [III.6.7](#).
- III.6.2.3 Policies must contain provisions to protect the confidentiality of:
 - a. those who report concerns, and
 - b. anyone against whom allegations are directed, while allegations are under investigation.

- III.6.2.4 The policy must also address mechanisms for protecting complainants from reprisal.

III.6.3 Origins of concerns or complaints

III.6.3.1 Concerns or complaints may arise from:

- a. animal care and use personnel, including researchers;
- b. other personnel, such as secretarial, maintenance, and security staff;
 - i. These persons are more likely to direct concerns to an animal care and use personnel, but they should be instructed to report concerns to the IACUC.
- c. employee “hotlines” or ombudsmen;
- d. the public;
 - i. The public are most likely to direct concerns or complaints to senior institutional representatives, who should promptly forward them to the IACUC Chair. See also [III.6.5.5.a](#).
- e. anonymous complainants; and
 - i. Anonymous complainants may or may not be institutional employees or students.
- f. the media.
 - i. Stories appearing in newspapers, or on television or radio, etc. may contain or evoke concerns about animal care and use.
 - ii. Such reports should be evaluated by the IACUC and, where appropriate, the IACUC should advise the institution to proactively address these concerns.

III.6.4 Methods for reporting concerns

III.6.4.1 Multiple avenues for reporting concerns must be available.

III.6.4.2 Names and contact information for multiple points of contact must be provided.

- a. Possible contacts include the IACUC Chair, the AV, the IO and others as appropriate.

III.6.4.3 Methods to report concerns about animal care and use must be readily accessible.

- a. Methods are to be posted at or near the entrances to animal facilities, in all animal procedure areas outside the vivarium, and when feasible on the institution's website.
 - i. Individuals should receive instruction on reporting perceived deficiencies in animal care or use. Refer to [IV.3.6.2.I](#).

III.6.4.4 Although written concerns may be more convenient to deal with, complainants may not be willing to submit concerns in writing.

- a. Requests for anonymity should be honoured, to the extent possible, regardless of the method of reporting.

III.6.5 IACUC response to concerns

III.6.5.1 The IACUC should acknowledge receipt of concerns when the complainant is known.

III.6.5.2 While specific methods for evaluating concerns about animal care and use may vary from institution to institution, all methods should include:

- a. a procedure for verifying concerns; and
 - i. Reviewing the complaint with the AV or designee.
 - ii. Depending on the nature of the concern, the IACUC office, legal counsel, and the person who submitted or fielded the complaint may be invited to participate.
- b. guidelines for effecting corrective measures, when appropriate.

III.6.5.3 The IACUC Chair is responsible for ensuring that concerns are addressed, but may delegate investigation to a subcommittee.

- a. If the IACUC Chair has, or is perceived to have, a conflict of interest, the IO should delegate the responsibility for assuring that the concern is addressed to another non-conflicted member of the IACUC.

III.6.5.4 Concerns may include situations or activities ranging from those in which animals are reported to be in immediate, actual or perceived jeopardy to those in which non-compliances are alleged but animals are not in apparent or immediate danger.

III.6.5.5 The course of action taken by the IACUC should be driven by the potential significance of the alleged situation.

- a. Conditions that reportedly jeopardise the health or well-being of animals must be evaluated as soon as reasonably possible.

III.6.5.6 Situations that may involve potential criminal activity should be reported to the police.

III.6.5.7 Issues that involve human safety must be reported promptly to occupational safety and health officials.

III.6.6 Outcomes

III.6.6.1 Institutions should develop self-regulatory policies and procedures to deal with verified non-compliance.

- a. Such policies and procedures are intended to ensure adherence to institutional and regulatory requirements.

III.6.6.2 The policies could adopt a range of remedial actions including, but not limited to, counselling and mandatory remedial training to specific monitoring of animal use, temporary revocation of animal use privileges, and suspension of a protocol. The adopted approach would depend on the severity of the non-compliance or deviation from accepted practices. Refer to [III.6.7.4](#) and [III.6.7.6](#).

III.6.6.3 The IACUC may suspend a protocol. This must be done at a convened meeting of a quorum of the IACUC, with the suspension vote of a majority of the quorum present.

- a. Suspensions must be reported to the IO, together with the reasons for the suspension. If applicable, the appropriate corrective action is also to be reported to the IO.
- b. The IO cannot over-rule an IACUC's decision to suspend a protocol.

III.6.7 Model of procedures for the investigation of animal care and use concerns and/or protocol non-compliance

III.6.7.1 For illustrative purposes, one model for considering concerns about animal care and use and/or protocol non-compliance is outlined below.

- a. This example can be adapted as needed by institutions.

III.6.7.2 Initial evaluation and actions

- a. Upon receipt of a concern the IACUC Chair should review the concern with the AV and/or one other member of the IACUC depending on the nature of the concern.
- b. After initial review of the complaint, the IACUC should determine whether it requires further investigation and immediate action, further investigation but no immediate action, or no action.
- c. Once the decision has been made, the IACUC should determine which individuals, and other institutional or non-institutional offices may require notification at this time.
- d. If immediate action appears warranted because animal or human welfare may be compromised, the IACUC should notify the IO, and if appropriate, occupational health and safety, and proceed with the necessary actions.
- e. Immediate actions may include:
 - i. veterinary medical intervention.
 - ii. stopping a research activity.

III.6.7.3 Investigation

- a. Should the IACUC determine that further investigation is required, the Chair, or another individual or subcommittee appointed by the Chair, should conduct the investigation and report back to the IACUC.
 - i. It is important to avoid actual or perceived conflicts of interest in this process.
- b. The IACUC should charge the designated person or subcommittee with its requirements for information gathering and determine an appropriate completion date.
- c. The nature of the information required will vary depending on the circumstances, but often involves:
 - i. interviewing complainants (if known), persons against whom allegations were directed, and pertinent staff;
 - ii. observing the animals and their environment; and
 - iii. reviewing any pertinent records (e.g. animal health records, protocol, and other documents).
- d. A report of the investigation's findings must be made to the IACUC.

- i. The report should summarise the:
 - a) concern(s),
 - b) condition of animals and their environment,
 - c) results of interviews, and
 - d) results of records and other document reviews.
- ii. The report should also contain:
 - a) supporting documentation such as correspondence, reports, and animal records;
 - b) conclusions regarding the substance of the concerns *vis-a-vis* requirements of the regulations, the NACLAR Guidelines, and institutional policies and procedures; and
 - c) recommended actions.

III.6.7.4 IACUC review of investigation report

- a. Upon receipt and evaluation of the investigation report, the IACUC may:
 - i. request further information;
 - ii. determine there was no evidence to support the concern or complaint;
 - iii. determine the concern or complaint was not sustained, but related aspects of the animal care and use programme require further review or other institutional programmes may require review; or
 - iv. determine the concern or complaint was valid.

III.6.7.5 Subsequent actions of the IACUC include:

- a. implementing measures to prevent recurrence, if appropriate. Such measures may include
 - i. changes in administrative, management or IACUC policies and procedures, and/or
 - ii. sanctions as described in [III.6.6.2-3](#) and [III.6.7.7](#);
- b. notifying the IO of its actions;

- c. notifying funding or regulatory agencies, as required; and
- d. notifying the complainant, any persons against whom allegations were directed, and pertinent programme officials (appropriate supervisory and management staff, the public affairs office, institutional attorneys, etc.).

III.6.7.6 Some institutions may develop policies and procedures that authorise the IACUC to impose sanctions on behalf of the institution.

- a. In other institutions, the IACUC may recommend actions to the IO for implementation.
- b. In some institutions, a combination of these approaches is used.

III.6.7.7 Some examples of sanctions include:

- a. letters of reprimand;
- b. mandating specific training aimed at preventing future incidents;
- c. monitoring by the IACUC or IACUC-appointed individuals of research, testing, or training involving animals;
- d. temporary revocation of privileges to provide animal care or to conduct research, testing, or training that involves animals, pending compliance with specific, IACUC-mandated conditions;
- e. permanent revocation of privileges to provide animal care or to conduct research, testing, or training that involves animals; and
- f. recommending to the IO that institutional sanctions be imposed.

III.6.8 Concerns unrelated to animal care and use

III.6.8.1 The IACUC may determine, either in its initial evaluation of a concern or as a result of investigation, that violations of non-animal-related institutional policies and procedures, may have occurred (e.g. scientific misconduct, misuse of monies, fraud, theft, etc.).

III.6.8.2 In such cases, those findings should be reported to appropriate institutional representative or committees for their consideration.

III.7 Record-Keeping and Communications

III.7.1 General

- III.7.1.1 The responsibility for IACUC record-keeping and communications should be clearly designated.
- III.7.1.2 Usually the IACUC secretariat is assigned this task.
- III.7.1.3 Records and other communications should be written clearly and precisely to ensure accurate interpretation.

III.7.2 IACUC Minutes

- III.7.2.1 Record of attendance
 - a. Members are marked as either present or absent.
 - b. It should be noted if an IACUC member leaves the meeting room during discussion of a protocol on which that member is a participant.
 - c. If the temporary absence of a member drops the number of members present below the quorum, this should be noted in the minutes.
 - d. Certain official IACUC actions require a quorum. Refer to [III.1.6](#) for quorum requirements.
- III.7.2.2 Activities of the IACUC that are to be recorded in the minutes include corrections or approval of the previous meeting minutes; presentation of programme, policy, facility and compliance reports; and decisions on policies, protocols, and amendments.
- III.7.2.3 Minutes must include, as a minimum, a summary of the key deliberation points leading to committee decisions and minority views.
 - a. Deliberations refer to the discussion and reasons leading to particular IACUC decisions.

III.7.3 Records

- III.7.3.1 The following records must be kept for at least three years; and must be accessible to NParks/AVS, and as appropriate to funding agencies:
 - a. Protocols submitted to the IACUC, even if approval was not granted or animals were not used. The records must show whether IACUC approval was given or not. However, when animals are used the protocols and amendments must be retained for the duration of the animal activity and for an additional three years after the end of the activity.

- b. Reports and recommendations from annual programme reviews and annual facility inspections.
- c. Reports of accrediting and/or other relevant agencies (e.g. AAALAC-International).
- d. Animal health records. These records are not usually maintained by the IACUC but are kept in the animal facility and are maintained by the applicable PIs and/or by veterinary staff.

III.7.4 Annual Report to NParks/AVS

- III.7.4.1 The research facility must prepare and submit an annual report to NParks/AVS. Refer to [II.9.3](#).

III.7.5 Collaborations or contract work

- III.7.5.1 A formal written agreement (e.g. contract, agreement, memorandum of understanding, etc.) must be developed for collaboration or contract work between two institutions' animal care and use programmes.
- III.7.5.2 At minimum, the written agreement should include clear descriptions of the following:
 - a. animal ownership,
 - b. responsibilities of each party, including animal care and occupational health and safety,
 - c. IACUC oversight and review of the protocol,
 - d. provision of veterinary care, and
 - e. post-approval monitoring.
- III.7.5.3 Both institutions should be made aware of any breach in protocol during a collaboration or contract work arrangement, and should discuss methods of communication and handling of breaches, non-compliance issues and/or unexpected outcomes.
- III.7.5.4 Based on the scope of the activities and accreditation status of the programmes, the institution owning the animals should consider the necessity of inspection of the other institution's animal care and use programme.

SECTION IV: TRAINING

IV.1 General

IV.1.1 These training guidelines are intended to assist the IO, IACUC, and others responsible for coordinating training programmes (e.g. training coordinators), in determining the scope and depth of such programmes.

IV.1.2 The IACUC is responsible for:

IV.1.2.1 evaluating the objectives of the training programme; and

IV.1.2.2 oversight of the training programme.

IV.1.3 The institution is responsible for financial support of the training programme.

IV.1.4 Anyone involved in the care and use of animals must be trained under these guidelines, including:

IV.1.4.1 personnel who do not handle live animals but perform support services (e.g. processing cages, washing equipment, cleaning facilities, disposing waste);

IV.1.4.2 personnel who perform husbandry (e.g. changing cages, providing feed and water, making daily observations, monitoring room environment);

IV.1.4.3 personnel who perform animal procedures (e.g. manipulations, restraint, administration of substances, collection of samples, surgery, harvest of tissues);

IV.1.4.4 personnel who supervise personnel described in [IV.1.4.1-3](#);

IV.1.4.5 veterinary personnel (e.g. veterinary technicians, veterinarians, AV);

IV.1.4.6 research personnel (e.g. PIs, researchers [including graduate and undergraduate students], research technicians);

IV.1.4.7 IACUC members and secretariat;

IV.1.4.8 service personnel; and

IV.1.4.9 instructors at tertiary institutions involved with using animals for teaching and/or educational purposes.

IV.1.5 For teachers at non-tertiary institutions, training is to be determined by the Ministry of Education.

IV.1.6 The training guidelines provide information on the basic training required for different types of personnel.

IV.1.6.1 It is recognised that further training may be needed depending on an individual's job scope and responsibilities.

IV.1.7 The methods for presenting materials will depend on the target audience, the objectives, the nature of the content, and the resources available.

IV.1.8 The training guidelines are not intended to cover all training on safety matters and handling biohazards.

IV.1.8.1 Comprehensive training on safety matters and the handling of biohazards is determined by each institution through its Occupational Health and Safety programme (OHSP). Refer to:

- a. Specific training requirements in [IV.2.1.2](#) and [IV.3.6.2.d](#) and
- b. [Section V: Occupational Health and Safety in Animal Care and Use](#).

IV.2 Animal Facility Personnel

IV.2.1 Personnel who do not handle live animals but perform support services (e.g. processing cages, washing equipment, cleaning facilities, disposing waste).

IV.2.1.1 In-house classes and on-the-job training should be conducted by proficient personnel (e.g. experienced laboratory animal technicians, managers, veterinarians).

IV.2.1.2 Topics to be covered:

- a. relevant regulations and guidelines;
- b. sanitation;
- c. responsibilities of care-takers and other animal facility personnel;
- d. personal hygiene, safety and protection, including PPE and ergonomics (e.g. repetitive stress injuries);
- e. handling of hazardous and waste materials;
- f. safe handling of relevant facility and laboratory equipment (e.g. autoclaves, washers, freezers, fumigation chambers etc.);

- g. compliance with SOPs and institutional policies (e.g. disaster plan, reporting animal welfare concerns etc.); and
- h. the institutional OHSP (refer to [V.3.2.a](#)).

IV.2.1.3 Personnel who do not handle live animals, but perform support services, should be encouraged to attend the didactic portion of the “Responsible Care and Use of Laboratory Animals” (RCULA) as described in section [IV.3](#).

IV.2.2 Personnel who perform husbandry (e.g. changing cages, providing feed and water, making daily observations, monitoring room environment)

IV.2.2.1 The laboratory animal manager, AV, director, their qualified alternate, or appropriately trained and experienced personnel should instruct on the following:

- a. all topics covered by personnel who perform support services – Refer to [IV.2.1.2](#);
- b. institutional policies for the care and use of laboratory animals; and
- c. awareness of local cultural and religious views on animals.

IV.2.2.2 Personnel who perform husbandry should be encouraged to achieve certification by the American Association for Laboratory Animal Science (AALAS) (see [AALAS, 2012-2018a](#) in [Appendix I: Section IV: Training](#)).

- a. Three levels are available:
 - i. Assistant Laboratory Animal Technician (ALAT);
 - ii. Laboratory Animal Technician (LAT); and
 - iii. Laboratory Animal Technologist (LATG).

IV.2.2.3 It is highly recommended that husbandry personnel be minimally certified at the ALAT level, but certification at the LAT and LATG levels should be encouraged.

IV.2.2.4 Husbandry personnel should be encouraged to attend other courses of relevance to their scope of work (e.g. husbandry for exotic animals, micromanipulation techniques, cryopreservation, tissue preservation etc.).

IV.2.2.5 Husbandry personnel must participate in the RCULA course (didactic and hands-on). Refer to [IV.3](#).

IV.2.3 Personnel who perform animal procedures (e.g. manipulations, restraint, administration of substances, collection of samples, surgery, harvest of tissues)

IV.2.3.1 The laboratory animal manager, AV, director, their qualified alternate, or appropriately trained and experienced personnel should instruct on the following:

- a. all topics covered by personnel who perform husbandry (refer to [IV.2.1.2](#) and [IV 2.2.1](#)); and
- b. protocol specific procedures and techniques relevant to each species to be used.

IV.2.3.2 These personnel must participate in the RCULA course (didactic and hands-on). Refer to [IV.3](#).

IV.2.4 Personnel who supervise those described in [IV.2.1](#) and [IV.2.2](#)

IV.2.4.1 Supervisors should be certified at least at AALAS LAT level.

IV.2.4.2 Supervisors should be encouraged to obtain certification from the Institute for Laboratory Animal Management (ILAM) (see [AALAS, 2018c](#) in [Appendix I: Section IV: Training](#)).

IV.2.4.3 These supervisors are also encouraged to be certified in the Certified Manager of Animal Resources (CMAR) programme (see [ICPM](#) in [Appendix I: Section IV: Training](#)).

IV.2.4.4 These supervisors must participate in the RCULA course (didactic and hands-on). Refer to [IV.3](#).

IV.2.5 Laboratory Animal Veterinarians

IV.2.5.1 The AV, and other laboratory animal veterinarians, should be encouraged to attend and participate in relevant conferences, scientific meetings and workshops.

IV.2.5.2 Obtaining board certification in laboratory animal medicine, such as through the American College of Laboratory Animal Medicine (ACLAM), is highly recommended (see [ACLAM](#) in [Appendix I: Section IV: Training](#)).

IV.2.5.3 These personnel must participate in the RCULA course (didactic and hands-on). Refer to [IV.3](#).

IV.3 Responsible Care and Use of Laboratory Animals (RCULA) Training

- IV.3.1 All personnel who care for and/or use laboratory animals, IACUC members and IACUC secretariat must attend the RCULA course unless exempted as described in [IV.3.11](#). Refer to [IV.1.4](#).
- IV.3.2 RCULA training provides an understanding of basic animal experimentation requirements, highlighting responsible animal handling, care and use, as well as responsibilities of researchers.
- IV.3.3 Only NParks/AVS-licensed animal research facilities at the institutes of higher learning (i.e. institutes of technical education, polytechnics and universities), hospitals from the public healthcare groups, and A*STAR may conduct RCULA courses.
 - a. Other animal research facilities must seek NParks/AVS' approval before conducting any RCULA training.
- IV.3.4 RCULA courses must comply with the NACLAR Guidelines.
- IV.3.5 RCULA courses should be conducted by appropriately qualified trainers.
- IV.3.6 RCULA course training components
 - IV.3.6.1 The RCULA course must consist of 2 training components:
 - a. didactic, and
 - b. relevant hands-on training.
 - IV.3.6.2 The RCULA didactic component may be delivered through lectures or online modules and must include:
 - a. introduction to NParks/AVS 'Rules on the care and use of animals for scientific purposes' (see [Animals and Birds \(Care and Use of Animals for Scientific Purposes\) Rules](#) in [Appendix I: Reference Materials: Legislations](#));
 - b. the NACLAR Guidelines and other relevant regulations and guidelines (e.g. Singapore Biosafety Guidelines for Research on GMOs, Biological Agents and Toxins Act (BATA), HBRA, etc.);
 - c. IACUC roles and responsibilities;
 - d. PI, IBC and IRB roles and responsibilities;

- e. the 3Rs (refer to [III.4.1](#)), ethical responsibilities, use of alternatives to live animals, and basic statistics to justify animal use numbers;
- f. occupational health and safety in animal care and use, including zoonosis, personnel protection equipment, laboratory animal allergies, biosafety and biohazards (refer to [Section V: Occupational Health And Safety In Animal Care And Use](#));
- g. animal handling, administration of substances and blood collection;
- h. basic laboratory animal anaesthesia and analgesia, including recognising pain and distress in animals;
- i. introduction to aseptic surgery, post-operative care and euthanasia;
- j. species-specific housing, space recommendations, social housing and environmental enrichment;
- k. animal biosecurity (e.g. vendor health report screening, health monitoring/ sentinel programme, etc.);
- l. record-keeping (e.g. animal identification systems, medical records, pre- and post-op care etc.);
- m. humane endpoints; and
- n. reporting of animal welfare concerns.

IV.3.6.3 RCULA hands-on training

- a. Using inanimate substitutes (e.g. training aids, simulators, cadavers etc.), is encouraged.
- b. A demonstration should be provided before trainees practise on animals.
- c. For certification in each of the commonly used species at an institution, hands-on training component should include:
 - i. providing information on basic biology of the species highlighting unique features (conducted before or during hands-on training);
 - ii. identification methods (e.g. cage cards, microchip, tattoo, ear and tail markings);
 - iii. gender determination;

- iv. restraint and handling;
 - v. common routes for administration of substances (e.g. oral, subcutaneous, intramuscular, intra-peritoneal, intravenous);
 - vi. blood sampling (e.g. from tail, limbs, ear, facial area);
 - vii. humane methods of euthanasia (e.g. carbon dioxide, barbiturates, non-chemical); and
 - a) Depending on species, discretion is to be exercised in determining if demonstration and/or hands-on exercises on euthanasia should be performed.
 - viii. harvesting tissues (e.g. spleen, liver, kidney, heart, lungs, brain).
- IV.3.7 It is important to note that personnel who have participated in the RCULA course may not be proficiently trained and may require additional supervision and training.
- IV.3.8 Refresher sessions for the didactic component (especially with regard to updates on regulations, guidelines and/or ethics) should be completed every 5 years.
- IV.3.9 Individuals with a hands-on practice lapse of 2 or more years should attend hands-on refresher training or assessment of competency.
- IV.3.10 Contents of the RCULA course should be reviewed, evaluated for effectiveness and updated regularly by the IACUC, in collaboration with the AV.
- IV.3.11 The IACUC may consider a waiver from RCULA for experts or instructors from overseas who have been engaged for short term teaching/demonstration sessions using live animals.
- a. Under these circumstances, it is recommended that veterinary staff provide oversight during the session.

IV.4 Assessing Additional Training Needs

IV.4.1 In evaluating protocols, the IACUC should assess whether personnel conducting procedures are appropriately qualified and trained in those procedures.

- a. For highly specialised procedures, the IACUC may look for formal documentation of mentor-assisted training of an individual.

IV.4.2 The IACUC must ensure that a policy is in place to ensure that training is completed by all personnel named in a protocol application before the protocol is approved by the IACUC.

IV.4.3 It is imperative that PIs include the duties, training, experience and qualifications for each person listed in the animal protocol application.

IV.4.4 If the IACUC deems personnel are not appropriately qualified, then the IACUC must require further appropriate training. As such, the IACUC may:

- a. mandate that the individual(s) complete relevant training before the technique(s), procedure(s) or manipulation(s) are approved,
- b. stipulate that the AV, or delegate, supervise the new technique, procedure, or manipulation if no relevant training module exists, or
- c. If there are no in-house personnel with the relevant expertise, the IACUC may seek a consultant for advice and training.

IV.4.5 To assist in decision making on appropriateness of an individual's qualifications and training, the IACUC should develop a list of items to be assessed.

IV.4.5.1 This could be a list of training items specific to procedures, and/or manipulations proposed described in each protocol; or

IV.4.5.2 It could be a list broad enough to cover all aspects of the institution's training requirements. For example:

- a. handling specific species,
- b. pain-relieving methods,
- c. surgical manipulations,
- d. aseptic technique,
- e. pain management,
- f. euthanasia, and

g. pre- and post-operative care.

IV.4.6 The IACUC also needs to assess that personnel listed on protocol applications have completed an occupational safety and health risk assessment and have been cleared to work on the protocol. Refer to [V.2.3](#).

IV.4.7 The IACUC must decide the level of training required of a PI not actually involved in the day-to-day manipulation and care of the animals.

IV.4.8 To prevent problems related to assessment of qualifications and training during protocol review, it is helpful if the IACUC determines any training needs during the protocol development and veterinary consultation.

IV.4.8.1 Discussion of new techniques, procedures, or manipulations at this time can provide the impetus for early training with demonstrated proficiency prior to protocol review. Such training experience should be documented and noted in the protocol application.

IV.4.9 Maintaining a database of all participants in the training programme will facilitate assessment of qualifications and training.

IV.4.9.1 With such a database, preliminary evaluation of an individual's expertise can be an administrative task performed by the IACUC secretariat or training staff.

IV.4.9.2 If a deficiency is noted, the PI can be notified early that there are pending training requirements so that protocol approval is not delayed.

IV.5 Addition of Personnel, Procedures or Manipulations, or New Species to a Protocol

IV.5.1 The IACUC must evaluate the qualifications and training of personnel added to an approved protocol, and ensure appropriate training before the personnel begin work on the protocol.

IV.5.2 When new or additional techniques, procedures, manipulations or species are proposed by the PI, the IACUC must assure itself that the personnel involved are qualified to perform the work and/or have been trained to work with the new species. Refer to [IV.4.4](#).

IV.6 IACUC Members and Secretariat

IV.6.1 IACUC members and secretariat must complete the didactic component of RCULA training.

IV.6.2 IACUC members and secretariat must also participate in relevant in-house training. In-house training topics should include:

- i. responsibilities and functions of IACUC members,
- ii. performing protocol reviews,
- iii. conducting programme reviews and facility inspections,
- iv. performing ongoing assessments of approved protocols, and
- v. addressing concerns involving animal care and use.

IV.6.3 At least 50% of IACUC members must receive formal training.

IV.6.4 Formal training must emphasise the roles and responsibility of the IACUC and provide other relevant information necessary for IACUC members to discharge their responsibilities.

IV.6.4.1 One excellent source for formal training is the SALAS Basic IACUC Training programme, which is endorsed by NParks/AVS.

- a. This is a full-day didactic training programme for new IACUC members, IACUC secretariat, and IOs, plus others responsible for their institution's animal care and use programme.

IV.6.4.2 Training for the IACUC secretariat may be obtained through the Public Responsibility in Medicine and Research (PRIM&R).

- a. PRIM&R holds annual IACUC conferences (see [PRIM&R, 2018b](#) in [Appendix I: Section IV: Training](#)) that cover topics relevant to animal care and use research ethics.
 - i. These topics include best practices in the field, understanding ethical requirements and dealing with challenges around the conduct of ethical animal research.
- b. IACUC Secretariat may choose to be certified under PRIM&R by undertaking the Certified Professional in IACUC Administration (CPIA) program and examination (see [PRIM&R, 2018a](#) in [Appendix I: Section IV: Training](#)).
 - i. The CPIA credential constitutes formal recognition of the IACUC secretariat staff member's broad knowledge of IACUC functions, and expertise about animal care and use programs.

IV.6.5 All IACUC members and secretariat should attend refresher courses to keep abreast with latest updates and reviews on the responsibilities of IACUC. Refer to [IV.3.8](#).

IV.6.6 Advanced IACUC training is encouraged for IACUC members, particularly the IACUC Chair and AV (see [AALAS, 2018b](#) and [AAALAC](#) in [Appendix I: Section IV: Training](#) for IACUC educational resources).

IV.7 Service Personnel

IV.7.1 Service personnel entering an animal facility should be accompanied by a member of the animal care staff.

IV.7.2 Service personnel should be provided with general information on the nature of facility, and any safety aspects prior to entering the animal facility.

IV.7.3 Service personnel should be briefed on special requirements to enter into rooms where large animals (e.g. NHPs, dogs) are housed or used, and/or any rooms where hazardous materials are used.

IV.8 Instructors and Teachers Involved in Using Animals for Teaching and/or Educational Purposes

IV.8.1 Tertiary level

IV.8.1.1 The person-in-charge of the class must attend the RCULA training.

IV.8.2 Non-tertiary level

IV.8.2.1 The care and use of animals at non-tertiary institutions are not covered by the NAACLAR Guidelines. Non-tertiary institutions are to refer to the Ministry of Education on the level of training required.

SECTION V: OCCUPATIONAL HEALTH AND SAFETY IN ANIMAL CARE AND USE

V.1 General

- V.1.1 Each institution must establish and maintain an occupational health and safety programme (OHSP) in animal care and use as an essential part of the animal care and use programme.
- V.1.2 The OHSP must be consistent with applicable legislations and guidelines, and should focus on maintaining a safe and healthy workplace with respect to the animal care and use programme.
- V.1.3 Applicable legislations and guidelines ([Appendix I: Reference Materials: Legislations](#) and [Appendix I: Section V: OHS](#)) include, but are not limited to the:
- a. [Animals and Birds Act \(Cap. 7\)](#),
 - b. [Biological Agents and Toxins Act \(BATA\) \(Cap. 24A\)](#),
 - c. [Human Biomedical Research Act \(HBRA\) \(Act 29 of 2015\)](#),
 - d. [Radiation Protection Act \(Cap. 262\)](#),
 - e. [Workplace Safety and Health Act \(WSHA\) \(Cap. 354A\)](#), and
 - f. Genetic Modification Advisory Committee's (GMAC) [Singapore Biosafety Guidelines for Research on GMOs](#).
- V.1.4 Where applicable, each institution must appoint an Institutional Biosafety Committee (IBC) and a Biosafety Co-coordinator/Officer (BSO), in accordance to GMAC Guidelines or BATA (Cap. 24A) requirements (see [GMAC](#) and [BATA](#) in [Appendix I: Reference Materials](#)).
- V.1.5 Where applicable, each institution must appoint an Institutional Review Board (IRB) in accordance to HBRA (Act 29 of 2015) (see [HBRA](#) in [Appendix I: Reference Materials: Legislations](#)).
- V.1.5.1 This is for the purpose of reviewing human biomedical research conducted under the supervision and control of the institution, and in accordance with other requirements as may be prescribed under applicable legislations and guidelines. Refer also to [III.3.1.2.a](#) and [III.3.1.2.b](#).
- V.1.6 The nature of the OHSP will depend on the facility, research activities, hazards and animal species involved.

V.1.7 An effective OHSP requires coordination between the research programme (as represented by the PI, the animal care and use programme, the AV, the IO and the IACUC), the occupational health and safety department of the institution, as well as a safety committee (or the IBC), as applicable.

V.1.8 The institution, PI and supervisors must protect the safety and health of staff or workers under his/her direction, as well as all persons who may be affected by their work. Their duties include the following:

- a. conduct risk assessments to identify hazards and implement effective risk control measures;
- b. ensure adequate safety measures for use of any machinery, equipment, article and/or process used at the workplace;
- c. develop and implement systems for managing emergencies;
- d. ensure workers are provided with sufficient instruction, training and supervision so that they can work safely; and
- e. ensure visitors/service personnel (where applicable) are made aware of potential hazards, are accompanied by a member of the staff and are properly protected.

V.1.9 It is the duty of everyone at the workplace to:

- a. follow the workplace safety and health system, safe work procedures and safety rules;
- b. not engage in any unsafe or negligent act that may endanger themselves or others;
- c. use the PPE provided to ensure safety while working;
- d. not tamper with, or misuse, equipment and hazardous material; and
- e. inform their supervisor of any unsafe practices or concerns of potential safety issues.

V.2 Hazard Identification and Risk Assessment

- V.2.1 An effective OHSP must ensure that health risks for each individual, associated with direct and indirect contact with animals used in research and teaching, are managed to an acceptable level.
- V.2.2 The institutional OHSP must identify potential hazards in the work environment and conduct a critical assessment of the associated risks, in accordance to MOM Workplace Safety and Health (Risk Management) Regulations (see [Workplace Safety and Health \(Risk Management\) Regulations](#) in [Appendix I: Reference Materials: Legislations](#)).
- V.2.3 An effective OHSP for animal care and use programs must also include protocol/procedure-based risk assessment and personal health risk assessment.
- V.2.3.1 Protocol/procedure-based risk assessment
- a. Identify and evaluate project/procedure-specific hazards and risks, and specify the appropriate controls to reduce the risks to minimal and acceptable levels.
- V.2.3.2 Personal health risk assessment
- a. An individual's personal health (e.g. immunosuppression) may put them at higher risk when working on specific animal research protocols.
 - b. A mechanism should be put into place to assess an individual's health on a regular basis in relation to the animal research protocols they will be involved in.
- V.2.4 The extent and level of personnel participation in the OHSP should be based on the:
- a. hazards posed by the animals and materials used;
 - b. severity or seriousness of the hazard;
 - c. exposure intensity, duration, and frequency of the hazard;
 - d. susceptibility (e.g. immune status) of the personnel; and
 - e. history of occupational illness and injury in the particular workplace.
- V.2.5 Health and safety specialists, with knowledge in relevant disciplines, should be involved in the risk assessment and development of procedures to manage such risks.
- V.2.6 Potential hazards include experimental hazards such as:
- a. biological materials (e.g. infectious agents, GMOs, zoonotic agents, toxins);

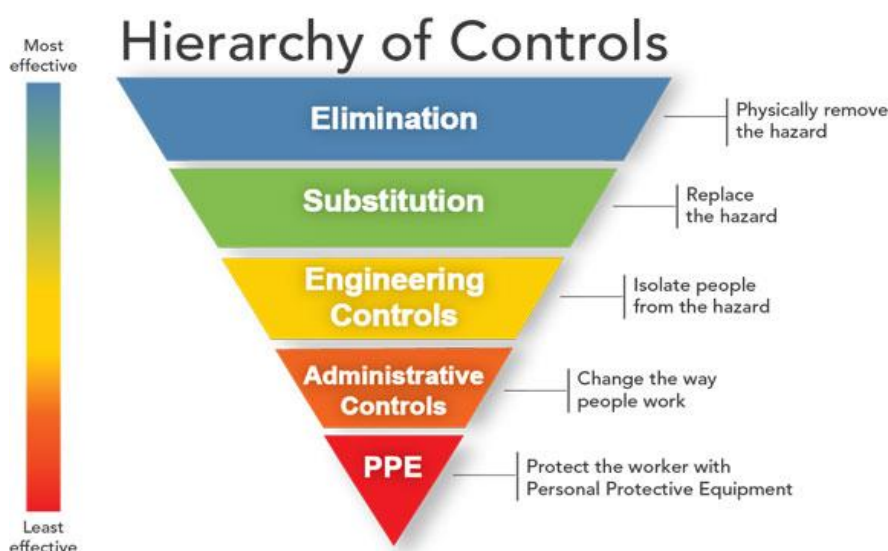
- b. chemical agents (e.g. toxic agents, cytotoxic agents, carcinogens, mutagens, teratogens, nanoparticles, novel compounds);
 - c. radiation (e.g. radionuclides, X-rays, lasers); and
 - d. physical hazards (e.g. needles, syringes).
- V.2.7 Risks associated with unusual experimental conditions, such as those encountered in field studies or wildlife research, should also be minimised.
- V.2.8 Other potential hazards that are inherent in or intrinsic to animal use (e.g. animal bites, exposure to allergens, chemical descaling and cleaning agents, wet floors, cage washers and other equipment, lifting, ladder use, zoonoses) should be identified and evaluated.
- V.2.9 Clear procedures must be established for reporting accidents, bites, scratches and allergic reactions.
- V.2.9.1 Medical care for such incidents must be readily available.
 - V.2.9.2 Accidents, dangerous occurrence or occupational disease should be appropriately reported, and in accordance with *WSHA (Cap. 354A)* or *BATA (C. 24A)* (where applicable) (see [WSHA](#) and [BATA](#) in [Appendix I: Reference Materials: Legislations](#)).
- V.2.10 Each institution must establish an emergency response plan that includes incident management.

V.3 Control and Prevention Strategies

V.3.1 A comprehensive OHSP should include a hierarchy of control and prevention strategies that is based on the identification of hazards and the assessment of risk associated with those hazards.

V.3.1.1 A sample hierarchy of controls (see [NIOSH](#) in [Appendix I: Section V: OHS](#)) to develop control and prevention strategies is shown in [V.3.1.2](#).

V.3.1.2 Priority should be given to control methods according to the following sequence: elimination→ substitution→ engineering controls→ administrative controls→ PPE; i.e. from the most effective controls to the least effective, whenever possible.



- a. Engineering controls include the appropriate design and operation of facilities, and use of appropriate safety equipment.
- b. Administrative controls include the development of processes and SOPs.

V.3.2 Managing risk requires that personnel:

- a. are trained, as appropriate, in the:
 - i. behaviour and handling of the animal species that they will work with;
 - ii. laboratory animal allergies;
 - iii. appropriate restraint techniques (may include chemical restraint or specialized restraint devices);
 - iv. project/procedure-specific risks;

- v. handling of hazardous materials (as defined in [III.4.6.1](#))
 - vi. handling of waste materials;
 - vii. record keeping and maintenance of records; and
 - viii. other considerations (e.g. precautions to be taken during pregnancy, illness, or immunosuppression) as appropriate to the risk imposed by their workplace;
- b. are aware of emergency and contingency plans;
 - c. are knowledgeable about the hazards in their work environment;
 - d. follow established procedures;
 - e. follow the approved protocol;
 - f. maintain good personal hygiene;
 - g. use appropriate PPE;
 - h. understand the proper selection and use of equipment;
 - i. participate in the institutional occupational health programme; and
 - j. report all injuries and follow established procedures for treatment.

V.4 Managing Animal Experimentation Involving Hazards

V.4.1 When selecting specific safeguards for animal experimentation with hazardous agents, careful attention should be given to procedures for:

- a. animal care and housing;
- b. storage and distribution/transportation of the agents;
- c. dose preparation and administration;
- d. body fluid and tissue handling;
- e. waste and carcass disposal;
- f. items that might be removed from the site (e.g. written records, experimental devices, sample vials); and

g. personal protection.

V.4.2 Institutions should have in place written policies and procedures governing experimentation with hazardous biological materials, chemical, radiation, sharps and other physical agents.

V.4.3 An oversight process (such as the use of a safety committee or an IBC) should be developed to involve persons who are knowledgeable in the evaluation and safe use of hazardous materials or procedures, and should include review of the procedures and facilities to be used for specific safety concerns.

V.4.3.1 Post-approval monitoring of approved protocols by the IACUC should include review of safe use of hazardous materials or procedures. Refer to [III.5](#).

a. A collaborative approach involving the investigator and research team, IACUC, AV, animal care technicians, and occupational health and safety professionals is necessary to enhance compliance.

V.5 Medical Evaluation and Preventive Medicine for Personnel

V.5.1 Part of the mission of the OHSP is to prevent occupational illnesses and injuries, reduce aggravation of pre-existing conditions, early recognition of health alterations due to occupational exposures, and the treatment and management of occupationally acquired illnesses and injuries.

V.5.2 Development and implementation of a medical evaluation and preventive medicine programme should involve input from trained health professionals, such as occupational health physicians and nurses.

V.5.3 Fitness to work with research animals in a particular research project will depend on the individual's health status and the health risks inherent in the nature of work.

V.5.3.1 A pre-employment/pre-placement health evaluation and/or a health history evaluation, to assess potential risks for individual employees, must be conducted.

a. The assessment of personal health risk should consider factors such as:

- i. the species and source of animals (and/or their tissues) to be worked with;
- ii. hazard exposure intensity;
- iii. hazard exposure frequency;
- iv. the hazardous properties of the agents;

- v. individual susceptibility;
 - vi. work activities (e.g. heavy lifting, repetitive work);
 - vii. fitness to use certain personal protective equipment (e.g. respiratory protection); and
 - viii. work in ABSL2, ABSL3 or ABSL4 areas.
- V.5.3.2 Periodic medical evaluations, as part of a medical surveillance programme, are generally advisable to monitor for changes in the individual's health, especially in certain occupational exposures such as animal husbandry workers; those who work with NHPs, bats or other potential reservoirs of zoonotic agents; or those who work with hazardous agents.
- V.5.3.3 A health evaluation and/or a health history evaluation should be carried out when job duties change (e.g. new species, new hazards) or there is a change in personal health.
- V.5.3.4 An appropriate immunisation schedule should be adopted.
- a. Animal care personnel, and other personnel who handle animals in the course of their work, should be immunised against tetanus.
 - b. Pre-exposure immunisation should be offered to people at risk of infection or exposure to specific agents such as rabies virus (e.g. if working with species at risk for infection) or hepatitis B virus (e.g. if working with human blood or human tissues, cell lines, or stocks).
 - c. Vaccination is recommended if research is to be conducted on infectious diseases for which effective vaccines are available.
- V.5.3.5 Pre-employment or pre-exposure serum banking is advisable only in specific circumstances, as determined by an occupational health and safety professional.
- V.5.3.6 Laboratory animal allergy has become a significant issue for individuals in contact with laboratory animals.
- a. The medical surveillance programme should promote the early diagnosis of allergies and include the evaluation of an individual's medical history for pre-existing allergies.
 - b. Personnel training should include information about laboratory animal allergies, preventive control measures, early recognition and reporting of allergy symptoms, and proper techniques for working with animals.

- c. PPE should be used to supplement, not replace, engineering or process controls.
- d. If PPE for respiratory protection is necessary, appropriate fit testing, medical fitness assessment, and training should be provided.

V.5.3.7 Zoonosis surveillance should be a part of an OHSP.

- a. Personnel must be instructed to notify their supervisors of potential or known exposures, and of suspected health hazards and illnesses.
- b. NHP diseases that are transmissible to humans can be serious hazards.
 - i. Because of the potential for exposure to Macacine herpes virus 1 (formerly Cercopithecine herpesvirus 1 or Herpes B virus), personnel who work with or handle biologic samples (blood and tissues) from macaques must have access to and be instructed in the use of bite, scratch, and mucous membrane exposure emergency care stations or kits.
 - ii. Careful evaluation, and appropriate post exposure treatment and follow-up should be implemented for injuries, eye splashes, or other mucus membrane exposure associated with macaques, their tissues or body fluids, or caging and equipment with which the animals have had direct contact.

V.5.3.8 Animal technicians, veterinarians, investigators, students, research technicians, maintenance workers, and others who have contact with NHPs or who have duties in NHP housing areas must be routinely screened for tuberculosis.

V.6 Facilities, Equipment and Monitoring

- V.6.1 The facilities required to support the OHSP will vary depending on the scope and activities of the programme.
- V.6.2 Facility design should preferentially use engineering controls and equipment to minimise exposure to anticipated hazards.
 - V.6.2.1 Such hazards may be related to the experiment, physical plant, animal-handling or animal husbandry.
 - V.6.2.2 Engineering controls and equipment that address the risk of ergonomic injury in activities such as the lifting of heavy equipment or animals should be considered.
 - V.6.2.3 Engineering controls should be used to limit or control personnel exposure to animal allergens.
 - V.6.2.4 The potential for repetitive motion injuries in animal facilities (e.g. maintenance of large rodent populations and other husbandry activities) should also be assessed.
- V.6.3 Noise control should be considered in facility design and operation (e.g. fans may be located on the roof top).
 - V.6.3.1 Separation of human and animal areas minimises disturbance to both.
- V.6.4 Special consideration should be given to the ventilation system, space arrangement and layout, support areas, traffic patterns and access to utilities and mechanical areas.
- V.6.5 The selection of appropriate animal housing systems requires professional knowledge and judgment, and depends on the:
 - a. nature of the hazards in question;
 - b. types of animals used;
 - c. limitations or capabilities of the facilities; and
 - d. design of the experiments.
- V.6.6 Experimental animals should be housed so that possibly contaminated food and bedding, faeces, urine, and other wastes can be handled in a controlled manner.
- V.6.7 Appropriate facilities, equipment and procedures should be used for bedding disposal.

V.6.8 Changing, washing, and showering facilities and supplies appropriate to the programme should be available. This is to ensure a high standard of personal cleanliness.

V.6.9 Where hazardous agents are used:

V.6.9.1 Design and safety features should be based on the level of risk posed by the agents used.

V.6.9.2 Special facilities and safety equipment may be needed to protect the animal care and investigative staff, other occupants of the facility, the public, animals, and the environment from exposure to hazardous agents used in animal experimentation.

- a. When necessary, these facilities should be separated from other animal housing and support areas, research and clinical laboratories, and human patient care facilities.
- b. These areas should be appropriately identified and access to them limited to authorised personnel.
- c. Safety equipment must be properly maintained and its function periodically validated.

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Appendix II: Additional Information on the Care and Use of Fish, Amphibians and Reptiles for Scientific Purposes

Fish

1. Given the diversity and number of species of fish, it is not possible to derive a set of comprehensive guidelines on housing, management and husbandry that can apply equally to all species of fish. However, some general principles for the care and use of fish in research can be used and these are briefly highlighted below. It is the responsibility of PIs to justify specific requirements of the species used, with appropriate reference to current literature and in consult with experts, if appropriate. These principles include:¹

Choice of species

2. The choice of fish species – whether marine, freshwater or brackish water – will determine much of the life support system needed. Ease of maintenance, space requirements and hardiness of the species are other considerations.

Sources, procurement and permits

3. The PI should obtain fish only from legal sources. See [II.6.1](#).
4. If wild-caught fish are to be used or field trials conducted, permission must be obtained from the relevant authorities, including the AVS/NParks and Public Utilities Board.
 - a. Catching / trapping fish from the wild or using them in field trials should be done according to acceptable prevailing standards.

¹ Adapted from two main publications:

1. DeTolla, L.J., Srinivas, S., Whitaker, B.R., Andrews, C., Hecker, B., Kane, A. S. & Reimschuessel, R., 1995. Guidelines for the Care and Use of Fish in Research. *ILAR Journal*, 37(4), pp. 159-173. [Online] Available at: <https://doi.org/10.1093/ilar.37.4.159>
2. Olfert, E. D., Cross, B. M. & McWilliam, A. A. eds., 2017. [Guide to the Care and Use of Experimental Animals \(Volume 1\). 2 ed.](#) Ottawa (Ontario, Canada): Canadian Council on Animal Care.

These publications (with the references contained therein) and a few others on the use of fish in research are listed as reference materials in [Appendix I: Reference Materials: General](#) and [Appendix I: Section II: Fish, Amphibians and Reptiles](#).

Transportation and handling

5. Care should be taken to reduce the stress associated with transportation. Withholding food for one to two days before transportation may be helpful to reduce fouling of the water, with a gradual return to normal feeding after transport. The PI and others working with fish should be proficient and equipped to handle the fish properly so as to minimise stress and injury to the fish. Where appropriate, chemical restraint should be judiciously and correctly used.
6. Fish should be handled as little as possible. Nets should be soft and only equipment that causes minimal damage to the skin of the fish should be used for handling fish.

Health Status

7. Before procuring fish, the AV should be involved in the assessment of the vendor to ensure the fish are healthy and fit for the intended trial. Where necessary, health screening can be conducted to ensure a clean, disease-free source of fish. Bleaching of embryos and rederivation may be required.

Acclimatisation

8. Proper acclimatisation should be done upon introduction of the fish to the facility.
9. Ideally the temperature of the water of the tank, in which the fish is kept on arrival, should be the same as that of the water in which the fish originated and was transported. However, as temperature change may be unavoidable, a gradual transition to the fish's preferred temperature should be made.
10. It is also important to pay attention to measures of water quality during acclimatisation.
11. During the acclimatisation period, the fish should be monitored frequently, and water parameters should be monitored more rigorously.
12. Fish should be disturbed as little as possible during their first few days in their new environment.

Quarantine

13. The AV must establish appropriate quarantine procedures.

Primary Containment

14. The tanks in which the fish are to be kept should be properly designed and constructed to meet the needs of the fish, and to ensure appropriate proper sanitation and protection of health.

Water Quality

15. Static systems require frequent cleaning of tanks and/or fish need to be kept at lower stocking densities in order to maintain acceptable water quality. For closed water systems, appropriate use of multiple filtration methods (e.g. biological filtration, mechanical filtration, chemical filtration and disinfection) should be used as appropriate to maintain good water quality.
16. Water parameters (i.e. temperature, hardness, conductivity, pH, dissolved oxygen, ammonia and nitrite levels) and frequency of monitoring, should be appropriate for the species and system, and demonstrable from literature and/or experts.
17. Water quality monitoring should be conducted according to the needs of the system and the user, to ensure that parameters remaining within acceptable range for the species of fish following activities such as feeding and water exchange.

Water temperature

18. The physiology and immune function of the fish are dependent on the water temperature, and it is crucial that water is maintained at the optimal range for the species of fish. Failure to do so can have a negative impact on health, well-being and survival of the fish. Design of tank rooms should take effects of down drafts from air-conditioning into consideration. Measures should be in place to prevent rapid fluctuations in water temperature.

Illumination

19. Both photoperiod and light intensity are important, and requirements vary among fish species.
20. Although most species do well with a cycle of 12 hours of light and 12 hours of darkness, 8 – 10 hours of light is generally adequate for most fish, while 12 – 14 hours of light is appropriate for tropical fish.

Stocking density and water flow

21. Different species of fish have different preferences and requirements for space. Allowing them as much space as possible may not necessarily be in their best interest. Optimum stocking densities depend on many factors, including water quality, species-specific natural behaviour, fish size, flow rates and temperature. At higher temperatures, the metabolism of the fish increases, thus raising the oxygen requirement of the fish while the oxygen level in the water decreases. Thus, the stocking density of fish may have to be lowered at higher temperatures, or supplemented with oxygen.
22. The water current should be appropriate to the species of fish.

Diet and feeding

23. Since nutritional requirements vary, an appropriate diet, frequency and method of feeding should be selected for the species.

Health programme, monitoring and disease control

24. The AV should ensure an appropriate health and disease monitoring programme is put in place to eliminate/minimise zoonotic agents, ensure the health of the fish, control spread of disease between tanks and to minimise effects of potential disease agents on research results.
25. Animal care staff and researchers must be trained to recognise health issues and report them to the AV to ensure adequate treatment/control of disease.
26. Appropriate safety procedures, including relevant PPE, must be in place and adhered to, to minimise the risk of contracting a zoonotic infection.

Analgesia, anaesthesia and invasive procedures

26. The AV must be consulted regarding the use of anaesthetics, analgesics, and invasive procedures.
27. During recovery from anaesthesia, the fish should be placed in a well-oxygenated, anaesthetic-free environment. To speed up recovery, forced ventilation can be accomplished by creating a flow of oxygenated water over the gills by moving the fish forward gently through the water. Animals must be closely monitored by a trained staff/researcher during induction, maintenance and recovery from anaesthesia.

Euthanasia

28. The prevailing AVMA Guidelines for the Euthanasia of Animals (see [AVMA Panel on Euthanasia](#) in [Appendix I: Section II: Euthanasia](#)) must be adhered to.

Dangerous species

29. The PI, in conjunction with the AV and the OHSP, should ensure appropriate safety measures are in place to minimise the risks of working with dangerous species.

Reptiles and Amphibians

1. As with fish, the large diversity of species of amphibians and reptiles make it challenging to derive a set of comprehensive guidelines for housing, care and management, husbandry and transport that can apply equally to all species.
2. The procedures used in the care and husbandry of amphibians and reptiles are typically different from other animals. It is the responsibility of the PI to justify specific requirements of the species used, with appropriate reference to current literature and in consult with experts as appropriate.
3. Should it be necessary to use wild-caught amphibians and reptiles in research, these animals may require different considerations from captive-bred and domesticated species.

4. Additional considerations² when using any amphibian or reptile:

- i) The PIs must have knowledge of all regulations pertaining to the animals under study, and must obtain all necessary permits for the proposed studies.
- ii) PIs must be familiar with the target species and its response to disturbance, sensitivity to capture and restraint, and, if necessary, requirements for captive maintenance to the extent that these factors are known and applicable to a particular investigation. Special concern should be shown for species known to remain with nests or young during certain seasons. Removal of individuals of species known to tend nests should be avoided during the nesting season, unless scientifically justified and approved by the IACUC.
- iii) Prior to removal of animals from the wild, every effort should be made to understand the population status (abundant, threatened, rare, etc.) of the taxa to be studied, and the numbers of animals removed from the wild must be kept to the minimum necessary to accomplish the goals of the study.

² Adapted from two main publications:

1. Herpetological Animal Care and Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists, 2004. Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research. 2 ed. Kansas: American Society of Ichthyologists and Herpetologists.

2. Canadian Council on Animal Care (CCAC), 2004. CCAC species-specific recommendations on: AMPHIBIANS AND REPTILES. [Online] Available at:

http://www.ccac.ca/Documents/Standards/Guidelines/Add_PDFs/Wildlife_Amphibians_Reptiles.pdf

These publications (with the references contained therein) and a few others references on the use of reptiles and amphibians in research are listed in [Appendix I: Section II: Fish, Amphibians and Reptiles](#). IACUCs and PIs may take reference from published references for specific guidelines and recommendations in the capture and management of reptiles and amphibians for scientific purposes.

Appendix III: Standards for Housing and Environmental Conditions¹

a) Mouse, Rat, Hamster, Guinea Pig, Gerbil

Animal	Weight/ gm	Floor area/ cm ²	Height/ cm
Mouse	<10	38	12
	Up to 15	51	12
	Up to 25	77	12
	>25 ^a	>96	12
Rat	<100	109	17
	Up to 200	148	17
	Up to 300	187	17
	Up to 400	258	17
	Up to 500	387	17
	>500 ^a	>451	17
Hamster	<60	64	15
	Up to 80	83	15
	Up to 100	103	15
	>100 ^a	>122	15
Guinea Pig	≤350	387	17
	>350 ^a	>651	17
Gerbil	-	116	15

^a Larger animals might require more space to meet performance standards.

¹ Tables a), b), c) and d) adapted from: National Research Council (US), 2011. Tables 3.2, 3.3, 3.6 and 3.1. In: [Guide for the Care and Use of Laboratory Animals](#). Washington (D.C): The National Academies Press, pg. 57, 59, 62-63 and 44.

b) Rabbit, Cat, Dog, Pigeon, Quail, Chicken, Non-Human Primate

Animal	Weight/ kg	Floor area/ m ²	Height/ cm
Rabbit	<2	0.135	35
	Up to 4	0.27	35 ^a
	Up to 5.4	0.36	40
	>5.4 ^a	>0.45	40
Cat	≤4	0.27	60
	>4 ^a	≥0.36	60
Dog^b	<15	0.72	-
	Up to 30	1.08	-
	>30 ^a	≥2.16	-
Pigeon^c	-	0.072	-
Quail^c	-	0.0225	-
Chicken^c	<0.25	0.0225	-
	Up to 0.5	0.045	-
	Up to 1.5	0.09	-
	Up to 3.0	0.18	-
	>3.0 ^a	≥0.27	-
Monkey^d (including the baboon)	Up to 1.5	0.20	76
	Up to 3	0.28	76
	Up to 10	0.4	76
	Up to 15	0.56	81
	Up to 20	0.74	91
	Up to 25	0.93	116
	Up to 30	1.4	116
	>30 ^a	2.32	152

^a Larger animals might require more space to meet performance standards. The cage height for rabbits should allow them to sit up without their ears touching the ceiling of the cage.

^b These recommendations might require modification according to body conformation of individual animals and breeds.

^c Cage height should be sufficient for the animal to stand erect and stretch its wings.

^d Callitrichidae, Cebidae, Cercopithecidae, and *Papio*. Baboons might require more height than other monkeys.

c) Goat, Sheep, Swine, Cattle, Horse, Pony

Animal	Weight/ kg	Floor area ^a / m ²	Height/ cm
Goat	<25	0.9 / 0.765 / 0.675	-
	Up to 50	1.35 / 1.125 / 1.017	
	>50 ^b	1.8 / 1.53 / 1.35	
Sheep	As for goat		
Swine	<15	0.72 / 0.37 / 0.37	-
	Up to 25	1.08 / 0.54 / 0.54	
	Up to 50	1.35 / 0.9 / 0.81	
	Up to 100	2.16 / 1.8 / 1.62	
	Up to 200	4.32 / 3.6 / 3.24	
	>200 ^b	5.4 / 4.68 / 4.32	
Cattle	<75	2.16 / 1.8 / 1.62	
	Up to 200	4.32 / 3.6 / 3.24	
	Up to 350	6.48 / 5.4 / 4.86	
	Up to 500	8.64 / 7.2 / 6.48	
	Up to 650	11.16 / 9.45 / 8.37	
	>650 ^b	12.96 / 10.8 / 9.72	
Horse	-	12.96	
	- (1 – 4 / pen)	72	
	≤200 (>4 / pen)	60	

^a Floor area per animal is given according to grouping sizes of 1, 2 to 5 and >5.

^b Larger animals might require more space to meet performance standards.

d) Temperature, Humidity, Ventilation and Lighting

The recommended temperature ranges for the different animals are as provided in the table below.

Animal	Dry-Bulb Temperature °C
Mouse, rat, hamster, gerbil, guinea pig	18 – 26
Rabbit	16 – 22
Cat, dog, non-human primate	18 – 29*
Farm animals and poultry	16 – 27*

*Animals housed in a sheltered outdoor environment with natural ventilation may acclimate to higher daytime temperatures experienced in the Singapore climate.

Fluctuations in temperature and humidity should be minimised.

The relative humidity should be 30 – 70% in an indoor housing environment for most mammalian species. Some species, such as amphibians, may require higher humidity.

The macro environment should be ventilated sufficiently to address heat loads, particulates, odours, and waste gases released from primary enclosures. A ventilation rate of 10 – 15 fresh air changes per hour in an indoor housing environment is an acceptable broad ventilation guideline for the macro environment. In some situations, the use of such a broad ventilation guideline might over-ventilate an enclosure that contains few animals or under-ventilate an enclosure that contains many animals. To determine more accurately the ventilation required, the minimal ventilation rate required to accommodate heat loads generated by animals can be calculated with the assistance of mechanical engineers. The minimal required ventilation is then determined by calculating the amount of cooling required to control the heat load expected to be generated by the largest number of animals to be housed in the enclosure plus any heat generated by non-animal sources and heat transfer through room surfaces.

In general, lighting should provide sufficient illumination for the animal's well-being while permitting good housekeeping, adequate animal inspection including for the bottom-most cages in racks, and safe working conditions for personnel. Light in animal holding rooms should provide for both adequate vision and neuroendocrine regulation of diurnal and circadian cycles. Lighting levels of 325 lux about 1.0 metre above the floor appear to be sufficient for animal care. For animals that have been shown to be susceptible to phototoxic retinopathy, light levels as low as 130 lux at cage level may be appropriate.

Appendix IV: Additional Information on the Care and Use of Non-Human Primates for Scientific Purposes

1. Non-human primates (NHPs) are recognised as having highly developed mental and emotional capacities, more so than most other animals used in biomedical research. In many ways, they are similar to humans and exhibit many similar external manifestations of emotions such as vocalisations, postures, gestures, and reactions.
2. While it is recognised that all species of animals used for scientific purposes should be provided with a physical environment conducive to their well-being, the greater complexity of NHPs requires greater emphasis on their psychological well-being through enrichment of their environment.
3. As with all animals used for scientific purposes, researchers and staff should acquaint themselves with the NHP's distinctive characteristics and needs. Every species of NHP is characterised by a specific behavioural repertoire. Ethograms or descriptive lists of species-typical behaviours, have been published for many species.
4. In Singapore, the NHP primarily used is the cynomolgus or crab-eating macaque, *Macaca fascicularis*, an Old World monkey. However, the common marmoset, *Callithrix jacchus*, a New World monkey has recently been introduced into research programs.
5. Researchers and staff should be able to recognise abnormal behaviour patterns and report them to the AV or designee. The AV should ensure that necessary steps are taken to treat or ameliorate them.

Despite the existence of variation between species of NHPs, it is possible to identify general categories of abnormal behaviours observed in captive species. Examples are summarised as follows:

- a. Stereotypical behaviours, such as self-biting, self-clasping, hair pulling, pacing, "saluting," and somersaulting;
 - b. appetitive disorders such as coprophagia (ingestion of animal's own faeces), urine drinking, and hyperphagia (excessive over-eating);
 - c. abnormal levels of activity, inactivity and depression-like behaviour;
 - d. deranged sleep patterns (increased night time activity) may be seen in subordinate animals;
 - e. abnormal social behaviours, such as, maternal neglect of infants, inappropriate sexual behaviour, and avoidance of social interactions.
6. Researchers and staff should have training or experience in animal cognition and perception. Frequent routine observation of every animal is important in order to provide optimal care and handling of the animals.

7. Researchers and staff should be able to recognise physical changes that may suggest chronic stress (alopecia, central obesity, wounds, etc.) and report any such findings to the AV.
8. Researchers and staff should be trained to recognise signs/symptoms of illness or disease in NHPs and to report such signs/symptoms to the AV.
9. NHPs that are not housed in accordance to international standards or treated inhumanely are likely to yield unreliable data due to the effects of behavioural stress. As with other laboratory animals, such stress can introduce unwanted variables.
10. Most NHP species are highly social, live in complex social groups and establish long-term bonds, although such bonds may not necessarily be permanent. Social isolation is likely to adversely affect individual animals. **Social Housing must be considered the default method of housing** unless otherwise justified based on social incompatibility, veterinary concerns, or scientific necessity approved by the IACUC. When groups or pairs are being formed, observers must adjust group composition so the units show minimal aggression.
11. NHPs form coalitions through which they establish their dominance ranks and compete for food and sexual partners. Removing a NHP from its group may disrupt the existing network of alliances and induce rank changes, which may be associated with vicious fighting resulting in injuries. Reintroduction of animals to groups following separation should be done with caution.
12. While enclosure size is important, the primary emphasis should be on providing the animal with the option for species-appropriate activities. Besides providing social peers, an animal's environment should also be enriched by providing food gathering activities, devices such as perches, shelves and swings and artificial appliances, such as audio-visual devices (radio, video, television). The latter appear to be more useful if the NHP can turn the equipment on and off at will. It has been reported anecdotally that NHPs are particularly fascinated by visuals depicting their natural environment, animals that are found in their natural habitat or videos of themselves.
13. Most NHPs show vertical flight reactions. This should be taken into account when arranging their housing. Attempts should be made to cater to their preferred vertical limits in the wild.
14. Because of the importance of vision to the NHP, cages should be positioned so that the NHPs can see conspecifics. Solid-sided caging prevents visual contact. If physical contact is possible, there must be assurance that the animals are compatible.
15. NHPs must never be housed in restraint devices, such as chairs, bags, slings, or on tables. Restraint devices should be used only to the extent necessitated by the nature of an experiment and the period of restraint should be no longer than is absolutely necessary to achieve the research objectives.
 - a. NHPs should be acclimatised to chair, or other restraint, through step-wise training and positive reinforcement prior to the start of the study.

- b. Criteria should be established for temporary or permanent removal of NHPs from a chair, or other restraint, if they fail to adapt.
- 16. Interaction between the NHP and the researcher or staff is encouraged but it should not be forced. The interaction, however, must not involve handling other than what is necessary for investigational procedures. Where single housing is necessary, interaction with people takes on added importance.
- 17. NHP familiarity with their handlers, surroundings and procedures can significantly reduce anxiety.
- 18. One of the most significant zoonotic diseases associated with macaques is Macacine herpes virus 1 (formerly Cercopithecine herpesvirus 1 or Herpes B virus). Appropriate procedures must be put in place for bites, scratches and mucous membrane exposures from macaque NHPs and potentially infective samples.
 - a. Personnel working with macaques must use appropriate PPE such as, body covering, head covers, foot covers, goggles or face shield, activity-appropriate mask, hand and arm covers.
 - b. Handling of potentially infective samples in the laboratory should be performed at the appropriate biosafety and containment level.
- 19. Humans can also transmit infectious diseases to NHPs, for example, measles and tuberculosis. It is recommended that staff should be vaccinated for measles and must routinely be screened for tuberculosis.
 - a. Anyone who is feeling ill should not enter NHP rooms or work with NHPs.
- 20. The selection of PPE for working with NHPs must be based on risk assessments, considering the hazards associated with the species of NHP and the activities being performed.

Appendix V: Signs of Animal Pain and Distress

Personnel involved in the care and use of animals for scientific purposes have the ethical and legal obligation under the prevailing Animals and Birds Act (Care and Use of Animals for Scientific Purposes) Rules (see [Animals and Birds Act](#) in [Appendix I: Reference materials: Legislations](#)) to reduce or eliminate pain and distress in the animals used. These actions should not interfere with research objectives and must be in line with the 3Rs principle. In order to do so, PIs and research staff, animal care staff, veterinary staff and IACUCs must be able to distinguish pain and distress in animals from their normal state. Pain and distress can then be relieved appropriately, and humane endpoints established.

Animals must be monitored by trained individuals for pain and distress as appropriate for the species, condition and procedure. Species vary widely in their response to pain. Critical to the assessment of the presence or absence of pain or distress is having the ability to distinguish between normal and abnormal animal behaviour. This is especially true when dealing with species that often exhibit pain and distress with only subtle changes in their behaviour. The following is a basic procedure for the development of a pain assessment that may serve until the development of species-specific pain assessment methods:

- Prepare a checklist of examinations to be undertaken, allow space for a general comment and perhaps an overall assessment tool (e.g. visual analogue scale (VAS) score sheet).
- Familiarise all staff who will be involved in the assessment with this checklist and any other assessment tools that will be used. Whenever possible, the same staff member should conduct each assessment of the same animal. Specific training must be provided for new or inexperienced staff.
- Begin by observing the animal without disturbing it. Assess the animal's response to the observer (the technician who routinely cares for the animal may be best to assess this).
- If the animal's behaviour changes markedly in the presence of the observer (e.g. as is the case with non-human primates, rabbits and guinea pigs), it may be more practical to assess post-operative or post-procedural behaviour by setting up a video camera or viewing panel.
- Examine the animal and assess its response to gentle palpitation or handling of any presumed painful areas (e.g. site of surgery, site of lesion) when practical.
- Weigh the animal, record its food and water consumption if possible, and examine the cage or pen for signs of normal or abnormal urination or defecation.
- Administer analgesic treatment if necessary, and repeat the assessment outlined above 30-60 mins after treatment to determine whether the drug and the dose administered have been effective. In the absence of certainty about the presence of pain, assessing the response to analgesic can be helpful.

Adapted from: National Research Council (US), 2009. BOX3-1: Pain Assessment Protocols. In: [Recognition and Alleviation of Pain in Laboratory Animals](#). Washington (D.C): The National Academies Press, p.49.

a) General pain assessment procedure:

- Pain and distress scoring is a method to convert subjective animal observations into a semi-quantitative scoring system which some have found to be helpful in assessing animal behaviour.
 - For example, grimace scales for mice¹, rats² and rabbits³ have been developed and supported by the National Centre for the Replacement & Reduction of Animals in Research (NC3Rs), and are now widely used in the animal research industry.

b) Potential signs associated with pain or distress in rats, mice and rabbits

- Potential signs associated with pain or distress in rats, mice and rabbits have been identified by the United States Office of Animal Care and Use (OACU) as follows.

¹ National Centre for the Replacement & Reduction of Animals in Research (NC3Rs), (n.d). *Mouse grimace scale*. [Online] Available at: <https://www.nc3rs.org.uk/mouse-grimace-scale>

² NC3Rs, (n.d). *Rat grimace scale*. [Online] Available at: <https://www.nc3rs.org.uk/rat-grimace-scale>

³ NC3Rs, (n.d). *Rabbit grimace scale*. [Online] Available at: <https://www.nc3rs.org.uk/rabbit-grimace-scale>

Potential signs associated with pain or distress in Rats, Mice and Rabbits⁴

Potential Signs	Mice	Rats	Rabbits
Decreased food and water consumption	x	x	x
Weight loss	x	x	x
Self-mutilation, gnawing at limbs	x	x	x
Rapid breathing	x	x	x
Opened-mouth breathing	x	x	x
Abdominal breathing	x	x	x
Grinding teeth		x	x
Biting/growling/aggression	x	x	x
Increase/decreased movement	x	x	x
Unkempt appearance (erected/matted/or dull hair coat)	x	x	x
Abnormal posture/positioning (e.g. head-pressing, hunched back, tucked abdomen)	x	x	x
Abnormal gait	x	x	x
Restless sleep			x
Tearing (including porphyria), lack of blinking reflex		x	x
Dilated pupils			x
Muscle rigidity, lack of muscle tone	x	x	x
Dehydration/skin tenting/sunken eyes	x	x	x
Twitching, trembling, tremor	x	x	x
Vocalization (rare)	x	x	x
Redness or swelling around surgical site	x	x	x
Increased salivation	x	x	x

⁴ Adapted from: The Office of Animal Care and Use, 2019. Table 1: Potential Signs Associated with Pain or Distress in Rats, Mice and Rabbits. In: *Guidelines for Pain and Distress in Laboratory Animals: Responsibilities, Recognition and Alleviation*, p.7. [Online] Available at: https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/b12_recognize_relieve_pain_distress_in_lab_animals_guideline.pdf

- Remember that:
 - The signs described here can be caused by conditions other than pain;
 - The signs may vary between animals of the same species, even after the same procedure; and
 - The signs will vary between strains and breeds

c) Post procedural pain potential⁵

- Whenever more than transient pain or distress is anticipated, pre-emptive measures should be taken to minimise or prevent the development of pain and/or distress.
- The extent and frequency of monitoring will depend on the level of post-surgical/procedural pain and/or distress anticipated and the chosen intervention strategy(s).
 - Animals undergoing a procedure known to produce no more than minimal/transient pain or distress may be adequately monitored by the daily observation of a trained personnel.
 - Whereas, the monitoring of an animal undergoing a procedure known to result in severe pain and/or distress may require more frequent monitoring by a team of trained individuals (e.g. trained animal care staff, technicians, veterinarians, investigators, etc.).
 - Animals undergoing pilot studies or procedures new to the investigator or facility may also require a higher frequency of monitoring and a team approach.

⁵ The analgesia and monitoring required may vary due to a number of factors; such as the invasiveness of the procedure, degree of tissue trauma, surgical time, skill of the surgeon, and the tissues or organs involved.

Post Procedural Pain Potential^{a, b, 6}

Minimal to Mild Pain^c	Mild to Moderate Pain^d	Moderate to Severe Pain^e
Catheter implantation	Minor laparotomy incisions	Major laparotomy/organ incision
Tail clipping	Thyroidectomy	Thoracotomy
Ear notching	Orchidectomy	Heterotopic organ transplantation
Subcutaneous transponder placement	C-section	Vertebral procedures
Superficial tumour implantation	Hypophysectomy	Burn procedures
Orbital sinus venotomy	Thymectomy	Trauma models
Rodent embryo transfer	Embryo transfer in non-rodents	Orthopaedic procedures
Multiple injections	Bone marrow collection	
Non-corneal ocular procedures	Corneal procedures	
Intracerebral electrode implantation		
Vasectomy		
Vascular access port implantation		
Craniotomy (periosteal pain)		
Superficial lymphadenectomy		

^a Table adapted from “Guidelines for the Assessment and Management of Pain in Rodents and Rabbits”. 2006, American College of Laboratory Animal Medicine

^b The analgesia and monitoring required may vary due to a number of factors; such as the invasiveness of the procedure, degree of tissue trauma, surgical time, skill of the surgeon, and the tissues or organs involved.

^c Post procedural pain relief for minimal to mild pain may be adequately addressed with pre-emptive analgesia, tissue infiltration with a long acting local anaesthetic, and a single dose of a long acting NSAID or mixed opioid agonist-antagonist, or other agent.

^d Post procedural pain relief for mild to moderate pain may be adequately addressed with tissue infiltration with a long acting local anaesthetic combined with one or more doses of a long acting NSAID and/or an opioid or other agent in addition to pre-emptive analgesic administration.

^e Post procedural pain relief for moderate to severe pain should encompass multimodal analgesia (e.g. combining a pure opioid agonist with a NSAID, tissue infiltration with a long acting local anaesthetic, etc.

⁶ Table adapted from: The Office of Animal Care and Use (OACU), 2019. Table: Post Procedural Pain. In: *Guidelines for Pain and Distress in Laboratory Animals: Responsibilities, Recognition and Alleviation*, p.8. [Online] Available at: https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/b12_recognize_relieve_pain_distress_in_lab_animals_guideline.pdf

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