UNIVERSITY OF
KWAZULU-NATAL

GUIDE

TO THE CARE & USE OF ANIMALS IN RESEARCH & TEACHING

Animal Ethics Committee of the University of KwaZulu-Natal

1. INTRODUCTION

The ability of scientists to enhance the well-being and our scientific knowledge of humans and animals depends directly on advancements made possible by research, much of which requires the use of experimental animals.[7] The replacement of living

animals by alternative methods is encouraged and will continue at an increasing pace, but the regrettable need for experiments on living animals will remain, principally for the following two reasons. First, it is impossible to imitate in any other way the immensely complex system of interactions between different organs that exist in every living animal; and second, in testing for the safety of medicines and other substances used in the home or work, specific tests on simplified systems will only detect the types of toxicity for which they have been designed, while there is an unlimited number of still unknown ways in which the substance may be poisonous and which can only be tested for by the administration to a living animal. Large numbers of animals are also used for non-invasive experiments which do not involve any kind of discomfort. These experiments include studies on the biology and ecology of animals for non-human purposes, often for conservation. Research is also done in the agricultural production field, such as feeding trials with farm animals to improve food production and food security for the local human population. The majority of experimental animal are purpose-bred and adapted to a laboratory or farm environment.

The vast benefits to both animals and man which have arisen directly from past animal research, and the reasonable expectation that such research will be of equal or greater benefit to all forms of life in the future, serves to justify the continuation of animal experimentation in general. However, the scientific community must recognise that they have both a scientific and ethical responsibility for the humane care of animals, and all who care for and use animals in research, testing and education must assume responsibility for their general welfare. It is especially important to recognise that the intent of research is to provide data that will advance knowledge of immediate or potential benefit to humans and animals. Scientists have developed, and should continue to develop scientifically valid adjuncture or alternative methods to animal experimentation.

Nothing in this *Guide* is intended to limit an investigator's freedom - indeed, obligation - to plan and conduct animal experiments in accordance with scientific and humane principles, and it should encourage scientists to seek improved methods of laboratory animal care.

2. PURPOSE[2]

This *Guide* has been prepared to give direction to the care and use of experimental animals by the research, teaching and technical personnel, undergraduate and postgraduate students of the University of KwaZulu-Natal (UKZN). Attention is directed to the important ethical, legal and scientific responsibilities associated with animal experimentation, and guiding principles are stated to assist individual animal users and University Schools in discharging their obligations in these regards.

Experimental animals include all living non-human vertebrates, their embryos and their foetuses, and Cephalopods that are captured or held in captivity. The immature forms of non-human vertebrates from the following stages of development are included:

i) mammals, birds and reptiles: from halfway through gestation or incubation periods;
ii) fish and amphibians: from the time at which they become capable of independent feeding.

A University Animal Ethics Committee composed of senior academics, a registered veterinarian, student, animal welfare, legal and nature conservation representatives has been appointed by the University Senate to monitor the use of living animals in teaching and research in University Schools. The members of this Committee have been selected on the basis of their qualifications and experience in the disciplines associated with experimentation. The functions of this Committee are twofold:

i) to assist researchers with any ethical problems which their teaching or research projects may raise;

ii) to access the protocols of all proposed experiments or research projects to ensure that they have been planned in accordance with accepted ethical and scientific practices.

3. HUMANE CONSIDERATIONS IN THE USE OF EXPERIMENTAL ANIMALS[2]

Man has a moral obligation to respect all animals and to have consideration for their capacity to be sensitive to pain, to suffer and to have a memory of such experience. The humane care and use of animal life is a prime responsibility of persons who use animals for experimental purposes.

It should be appreciated that humans are not naturally endowed with an instinct to be kind to animals. Although based in part on the conditioning of inborn emotional responses, the evolution of humaneness is associated principally with the psychological and social development of individuals. Although humaneness is part of our culture this does not always suffice to protect the interests of animals when they are used in research to promote human welfare. There is nothing in the process of becoming an animal technologist or a scientist who experiments with animals that ensures humaneness unless their education includes attention to this problem[3.] Animal experimentation should therefore be considered to be a discipline for which a special education programme is necessary.

Many animal experiments such as those concerned with nutritional studies, reproduction, animal behaviour, or parasitic infestation can be conducted without inflicting any pain on an experimental animal. The same cannot be said of many physiological and surgical experiments in which operative procedures have to be performed. Despite good anaesthetic and surgical techniques and post-operative care, the procedures are unavoidably unpleasant for the animal subjects on which they are performed. The use of large numbers of small animals for biological assay procedures, drug and vaccine development and testing, and toxicity testing in which substances are administered orally, topically or parenterally often produce extreme discomfort and death. In many instances food and drug standards are maintained by biological testing which is mandatory in terms of legislation.

No scientist who is well informed can therefore honestly claim that laboratory animals never experience pain and discomfort, or that the quality of animal care and treatment in all laboratories and institutions is optimal. The ethical position of the scientific community is weakened if it exhibits ignorance of these facts or attempts to conceal them.

In the context of animal experimentation, humaneness is concerned with reducing the sum total of fear, discomfort and pain that may be caused to laboratory animals.[1,4]

Such discomfort and pain may be caused directly by an experimental procedure or may be a contingent factor arising from methods of animal procurement, transportation, nutrition, handling and restraint or from exposure to injury, communicable disease or parasitism. Contingent inhumanity is almost always detrimental to the objectives of an experiment since it introduces psychological and physiological disturbances which are likely to confuse almost any biological investigation.

In reducing the sum total of discomfort and pain in an animal experiment both quantitative and qualitative aspects need to be considered. These concern the number of experimental subjects and the severity of distress and pain which a procedure may produce in the individual animal subject.

Reduction of the sum total of discomfort and pain may be accomplished by applying the following four approaches:

i) Replacement of living animals where possible with scientifically valid alternative methods using insentient material.

ii) Reduction of the number of animals in an experiment to the minimum number required to obtain information of a given degree of precision by the right choice of strategies in the planning and performance of research and by the application of statistical design and analysis.

iii) Refinement of experimental techniques by:

   a) improvisation and the application of technological advancements in order to reduce the severity of an experimental procedure on those animals which still have to be used;

   b) the elimination of contingent inhumanity which is associated with animal handling and husbandry in an animal facility.

iv) Responsibility of the user for the care and welfare of the animals under his/her control.

Principles regarding the ethics of animal experimentation are described by the *Canadian Council on Animal Care*[5] and concern the humane treatment of animals. The University ascribes to these principles which are as follows:

1. For experimental purposes, animals should be used only if the researcher's best efforts to find an alternative have failed, and should entail a continuing review of the literature.[6] Scientists have a responsibility to share with other scientists research information, methodology and experience.
2. Those using animals should recognise the need to use the best methods on the smallest number of appropriate animals required to obtain valid information.[7] Proposed experiments must be justifiable in terms of the declared objectives. The experimental design must offer every practicable safeguard to the animal.
3. There must be reasonable expectation that studies involving animals will contribute significantly to knowledge which may eventually lead to the protection and improvement of the health and welfare of either humans or animals, or the conservation of the latter. Expert opinion must attest to this premise in particular before undertaking the following procedures, which are restricted:

i) Prey killing and fighting.

ii) Burns, freezing injuries, fractures, and other types of trauma investigation. These require anaesthesia during procedures, and must include acceptable veterinary practices for the relief of pain.

4. Animals are not to be subjected to unnecessary pain or distress. Their physical and mental well-being are paramount.

5. If pain or distress are necessary concomitants to the experimental study, these should be minimised both in intensity and duration.

   An informed assessment of the probable degree of pain and its duration is required, in order to relate these to acceptable, or unacceptable, limits. Investigators, animal care committees, grant review committees and referees must be especially cautious in evaluating the proposed use of the following procedures:

   a) experiments that involve withholding pre- and post-operative pain-relieving medication;

   b) paralysing and immobilising experiments where there is no reduction in the sensation of pain;

   c) electric shock as negative reinforcement;

   d) extreme environmental conditions such as low or high temperatures, high humidity, modified atmospheres, etc., or sudden changes therein;

   e) stress and pain research.

6. An animal observed to be experiencing severe, unrelievable pain should immediately be humanely killed, using a method providing initial rapid unconsciousness.

7. Acute (non-recovery) procedures involving animals anaesthetised and insensitive to pain during an entire study are considered acceptable, as are studies which involve no pain or distress.

8. The following experimental procedures are known to inflict excessive pain and are thus unacceptable:

   a) utilisation of muscle relaxants or paralytics (curare and curare-like) alone, without anaesthetics, during surgical procedures;

   b) traumatising procedures involving crushing, burning, striking or beating in unanaesthetised animals.

9. Studies such as toxicological and biological testing, cancer research and infectious disease investigation may, in the past, have required continuation until the death of the animal. However, in the face of distinct signs that such processes are causing irreversible pain or distress, alternative end points should be sought to satisfy both the requirements of the study and the needs of the animal.

10. In test procedures, cost and ease of application should not overrule the need to prevent pain and distress.

11. Experiments involving the withholding of food or water should entail only short-term deprivation and have no detrimental effect on the health of the animal.

12. Physical restraint should only be used after alternative procedures have been fully considered and found inadequate. Animals so restrained must receive exceptional care and attention, in compliance with species specific and general

requirements as set forth in the *"Guide to the Care and Use of Experimental Animals"*.[5]

13. Painful experiments or multiple invasive procedures on an individual animal, solely for the instruction of students in the classroom or for the demonstration of established scientific knowledge in e.g., exhibits, conferences or seminars, cannot be justified. As a replacement, audio-visual techniques should be employed to convey such information.

The above principles should be applied in conjunction with those outlined in this *"Guide to the Care and Use of Animals in Research and Teaching"*, as prepared and distributed by the Animal Ethics Committee of the University of KwaZulu-Natal.

4. PAIN AND THE CLASSIFICATION OF PAIN

All animals from protozoa to mammals are living organisms that respond to stimuli. These stimuli may be extreme or prolonged and cause discomfort, distress/stress or pain to the animal. The concept of pain and distress in animals is subjective and difficult to define and the human assessment therefore tends to be anthropomorphic. An author on pain states:

Until further progress is made in assessing the nature of pain in animals, it should be assumed that if a procedure is likely to cause pain in man, it will produce a similar degree of pain in animals. (P.A. Flecknell; The Relief of Pain in Laboratory Animals, Lab Animal, 18: 147-160, 1984)

It is, however, important to recognise the three states of suffering which may be described as follows:

Discomfort

characterised by negative signs of poor condition, torpor, diminished appetite.

Stress/Distress

is an undesirable physical or mental state resulting from pain, anxiety, or fear. Its acute form may be relieved by tranquillisers. Sustained distress, however, requires environmental change and behavioural conditioning and does not respond to drug therapy.

Pain

Acute Pain resulting from a traumatic surgical or infectious event that is abrupt in onset and relatively short in duration. It is generally alleviated by analgesics.

Chronic Pain results from a long-standing physical disorder or emotional distress that is usually slow in onset and has a long duration. It is seldom alleviated by analgesics but frequently responds to tranquillisers combined with environmental and behavioural conditioning.

An animal in pain, regardless of species, usually displays one or more of the following signs:

- attraction to the area of pain
- increased skeletal muscle tone
- altered electroencephalogram response

- increased blood pressure and heart rate
- pupillary dilation
- change in the respiratory pattern

It is the view of the Animal Ethics Committee that animals always be given the benefit of any doubt concerning pain relief. The following principles apply:

1. Every animal user shall take effective precautions to prevent or reduce to a minimum any pain or other distress or discomfort in the animals used.
2. Procedures which are likely to cause fear, stress, discomfort or pain should be performed under anaesthesia unless the effect of the procedure on the animal's well-being is less than that caused by anaesthesia.
3. At the end of an experiment, animals should be euthanased if they are likely to remain in discomfort and pain.
4. In no case shall any animal be subjected to severe pain which endures or is likely to endure.
5. Experiments which cannot be performed without unrelieved pain and distress should be abandoned.

In an attempt to assess the degree of pain inflicted on research animals, a pain classification system developed by the *Canadian Council on Animal Care* is used as a reference. This system categorises pain on the degree of invasiveness in animal experiments. The researcher is requested to refer to this pain classification system which should be applied to all research as part of the protocol design and review process.

5. CATEGORIES OF INVASIVENESS IN ANIMAL EXPERIMENTS

5.1 Studies or experiments on most invertebrates, or on non-entire living material

These might include: tissue culture, tissues obtained at autopsy, necropsy or from the slaughterhouse; eggs, protozoa and related single celled organisms; studies or experiments involving containment, incision or other invasive action on metazoa. It is acknowledged that cephalopods and some other higher invertebrates have nervous systems as well developed as some vertebrates and therefore Categories of Invasiveness B, C, D, and E may apply.

5.2 Studies or experiments on vertebrates causing little or no discomfort or stress

These might include: holding of animals captive for observation or physical examination; blood sampling; injection of non-toxic material by the following routes: intravenous, subcutaneous, intramuscular, intraperitoneal, or oral, excluding intrathoracic or intracardiac; acute non-survival experiments in which the animals are completely anaesthetised and do not regain consciousness; standard methods of euthanasia that induce rapid unconsciousness; short periods (few hours) of food and/or water deprivation.

5.3 Studies or experiments on vertebrates involving minor stress or pain of short duration

These might include: cannulation or catheterisation of blood vessels or body cavities performed under anaesthesia; minor surgical procedures under anaesthesia, such as biopsies, laparoscopy; short periods of restraint consistent with minimal distress; overnight food and/or water deprivation; behavioural experiments on awake animals that involve short-term stressful restraint. These would not cause significant change in coat appearance, ocular or nasal discharges, abnormal respiratory or cardiac rate, reduction of faecal or urinary output, isolation or crowding.

Comment: During or after Category C studies animals must not show self-mutilation, anorexia, dehydration, hyperactivity, increased recumbency or dormancy, increased vocalisation, aggressive-defensive behaviour or demonstrate social withdrawal and self-isolation.

5.4 Studies or experiments on vertebrates that involve moderate to severe distress or discomfort

These might include: major surgical procedures conducted under anaesthesia, permitting recovery, with adherence to acceptable veterinary practices, adequate post-operative analgesia, fluid therapy and required veterinary nursing practices; exposure of animals to noxious stimuli for periods not above the minimal level required to demonstrate the required clinical effect; prolonged (several hours or more) periods of physical restraint applied in compliance with standard guidelines; induction of behavioural stresses such as maternal deprivation, aggression, predator-prey interactions, procedures which alter perceptual or motor functions which consequently affect locomotion and behavioural activity; immunisation employing Freund's complete adjuvant administered subcutaneously or intramuscularly; induction of an anatomical or physiological deficit that will result in pain or distress; application of noxious stimuli from which escape is impossible; procedures that produce pain in which anaesthetics are not used, such as toxicity testing with death as an end point; production of radiation sickness; certain injections, and stress and shock research that would result in pain approaching the pain tolerance threshold.

Comment: Animals used in Category D studies should not have signs of prolonged clinical distress, such as marked abnormalities in behavioural patterns or attitudes; lack of grooming, dehydration, abnormal vocalisation, prolonged anorexia, circulatory collapse, or decreased cardiac activity, increased signs of infectious processes (peritonitis, pleurisy, pneumonia, diarrhoea, etc.). If the clinical abnormalities cannot be alleviated, the animals should be destroyed using an acceptable method of euthanasia.

5.5 Procedures that involve inflicting severe pain near, at, or above the pain tolerance threshold of unanaesthetised, conscious animals

Such studies may not be confined to surgical practices, but may include exposure to noxious stimuli or agents whose effects are unknown; intradermal or foot pad injection using Freund's complete adjuvant; completely new biomedical experiments which have a high degree of invasiveness; behavioural studies about which the effects of the degree of distress are not known; use of muscle relaxants or paralytic drugs without the use of anaesthetics; burn or trauma infliction on unanaesthetised animals; a euthanasia method not approved by the South African Veterinary Council or related bodies.

Comment: Category E experiments are considered highly questionable or unacceptable, irrespective of the significance of anticipated results. Many of these procedures are specifically prohibited because of conflict with internationally accepted "Ethics of Animal Experimentation".

6. NATIONAL GUIDELINES FOR THE HUMANE TREATMENT OF ANIMALS USED IN RESEARCH, EDUCATION, DIAGNOSIS AND TESTING OF DRUGS AND RELATED SUBSTANCES IN SOUTH AFRICA

The Department of Agriculture released a *National Code of Ethics* to govern the use of experimental animals in testing, teaching and research in August 1990. Although not regulated by law at this stage, all users and producers of experimental animals are requested to abide by its principles. The University of KwaZulu-Natal acknowledges this *National Code of Ethics* and is committed to its intention and content.

The *Code* is reproduced in its entirety and is as follows:

6.1 PREAMBLE

6.1.1 The optimal care and use of experimental animals is absolutely essential and in the interest of both the animals and research. There are two main reasons for the proposition. Firstly, it is expensive to use animals in experiments. Experimental animals which are housed in poor facilities or are suffering from disease produce poor, unreliable and unrepeatable results, and are wasteful of resources. Secondly, civilised societies should be mindful of humane aspects. Proper care and use promotes the welfare of animals and contributes greatly to the attainment of high ethical and humane standards.

6.1.2 Man has an obligation to respect animals and to appreciate that they are sensitive to pain, respond to stress and may remember such experiences.

6.1.3 The use of animals in education, research and testing of drugs is important to the biological sciences and contributes to man's knowledge of life and nature.

6.1.4 Every effort should be made to refine, reduce and replace animal experimentation.

6.1.5 The use of animals in experiments shall be fully justified and discomfort, stress and distress should be kept to a minimum or, ideally, avoided.

6.2. DEFINITIONS

6.2.1 ***Experimental animals***

The term "experimental animals" encompasses all non-human vertebrates, vertebrate foetuses and embryos and members of the class Cephalopoda (i.e. all animals that have, or have the potential for developing a nervous system capable of perceiving painful stimuli).

6.2.2 ***Animal experiment***

An 'animal experiment' is any procedure which uses experimental animals for one or more of the following:

(a) the advancement of knowledge;
(b) to test a hypothesis;
(c) to supply a product;
(d) to provide organs, tissues or sera;

(e) to act as a host;
(f) to impart or demonstrate existing knowledge;
(g) to teach or learn surgical and other techniques;
(h) to fulfil statutory requirements for testing or collecting data on any substance or product; and
(i) to make audio-visual recordings of any of the above.

6.3. PERSONNEL

6.3.1 Control of the use and care of animals undergoing experiments shall be the responsibility of an institutional Animal Ethics Committee (see 7.1 below).

6.3.2 The Animal Ethics Committee shall ensure that the clinical care of experimental animals is the responsibility of a veterinarian.

6.3.3 Supervision of housing, feeding and breeding (animal husbandry) shall be the responsibility of a person who, irrespective of any other training, has specific qualifications or expertise in this area.

6.4. USE OF ANIMALS IN EXPERIMENTS

6.4.1 The principal object of the use of animals shall be to obtain useful results and scientific information of high quality for the benefit of man and animals.

6.4.2 Use of animals shall be thoroughly and scientifically planned and based on knowledge of the problem under study and so designed that the expected results will justify the execution of the experiment.

6.4.3 All efforts shall be made to reduce the use of animals to a minimum. Animals shall not be used if a reasonable and valid alternative exists.

6.4.4 Experiments that cause protracted, intense pain from which the animal cannot escape, shall not be performed.

6.4.5 Clinical care of animals, before, during and after application of experimental procedures, shall be of a high standard and according to accepted veterinary practice and shall aim to avoid, minimise or end pain or any other harmful effects which may arise from the procedure. No experimental animal shall be disposed of before absolute certainty exists that death has occurred.

6.4.6 The veterinarian shall possess an overriding discretion as to when an animal or animals shall be killed or withdrawn from an experiment for humane reasons.

6.4.7 The husbandry care of animals shall comply with recognised standards. Special attention shall be given to aspects such as regular feeding, adequate and clean water, good environmental hygiene, adequate ventilation and elimination of excessive heat, cold and noise from the environment of the animal. Precautionary measures shall be taken to prevent diseases, injuries, overcrowding and stress factors and to protect the animals from endo- and ectoparasites.

6.4.8 No animal shall be subject to more than one procedure that causes significant pain.

6.5. FACILITIES

6.5.1 The cages in which animals are held and the premises in which the cages are housed shall comply with recognised standards (see Schedule C).

6.5.2 Where wild animals are trapped in their natural habitat for research purposes the cages shall comply with standards prescribed by nature conservation authorities.

The cages shall be visited daily to prevent the animals being left for long periods without food and water and to prevent unnecessary discomfort.

6.6. TRANSPORTATION

6.6.1 Experimental animals shall be transported in accordance with recognised standards and regulations (see Schedule B).

6.6.2 On arrival at an airport, seaport, railway station or other destination the experimental animals shall be immediately unloaded and transported to suitable permanent housing.

6.6.3 Appropriate veterinary care shall be given to experimental animals found to be in a diseased, injured or other poor state during travel or on arrival.

6.7. CONTROL AND INSPECTION

6.7.1 All institutions or bodies using animals for purposes referred to in paragraph 2.2 shall establish Animal Ethics Committees which shall

(a) be properly constituted;

(b) carry out the responsibilities and duties as set out in Schedule A;

(c) ensure that these guidelines are followed and applied;

(d) keep records of the type and the number of animals used in each experiment; and

(e) ensure that quantifiable norms and standards are adhered to (Schedules B and C).

SCHEDULE A

7. ANIMAL ETHICS COMMITTEE (AEC)

7.1. Objective

An Animal Ethics Committee shall control the use and care of experimental animals.

7.2. Membership

An AEC is constituted by, and shall report to the management of the Research Institution. The Committee shall preferably include persons from each of the following categories:

7.2.1 Established researchers who have experience of the use of animals in experiments.

7.2.2 A registered veterinarian.

7.2.3 Persons not engaged in research representing recognised animal welfare organisations and appointed by mutual agreement between the institution and the organisation.

7.2.4 Persons not engaged in research and not associated with the institution or a recognised animal welfare organisation.

7.3. Responsibilities

7.3.1 to establish and enforce institutional codes of practice for the use and care of animals for teaching and research;

7.3.2 to record information on the type of experiments done;

7.3.3 to preview and review all animal experiments performed in the institution;

7.3.4 to ensure that proposed experiments are justified and feasible;

7.3.5 to ensure that an animal, or the animal chosen, is the best model and that no reasonable alternative method can be applied;

7.3.6 to ensure that the means and aims of experiments meet current scientific, moral, ethical, legal and institutional requirements;

7.3.7 to ensure that pain, discomfort, stress or distress is minimised or eliminated;

7.3.8 to monitor the progress of experiments; and

7.3.9 to ensure that the researcher is adequately qualified to perform the experiment.

7.4. Duties

In order to meet the responsibilities outlined in paragraph 3, the duties of an AEC shall include the following:

7.4.1 to meet regularly;

7.4.2 to create mechanisms that provide for -

7.4.2.1 the stringent review of experiments;

7.4.2.2 acceptable standards of clinical care of animals;

7.4.2.3 acceptable standards of husbandry care of animals;

7.4.2.4 the rapid stopping of experiments; and

7.4.3 to report regularly to the management of the institution.

SCHEDULE B

8. SOME RECOGNISED STANDARDS, BODIES AND PUBLICATIONS ON THE CARE AND USE OF LABORATORY ANIMALS

1. Institute for Laboratory Animal Resources, National Research Council, 2101 Constitution Avenue N.W., Washington, DC 20418. *Guide for the Care and Use of Laboratory Animals* (revised 1985).
2. Canadian Council on Animal Care, 1105-151 Slater Street, Ottawa, Ontario, KIP 5H3. *Guide to the Care and Use of Experimental Animals*, volumes I and II (1980).
3. Biological Council, Institute of Biology, 41 Queens Gate, London SW75 HW. *Guidelines on the Use of Living Animals in Scientific Investigations* (1984).
4. Council for International Organizations of Medical Services (CIOMS). W.H.O. Distribution and Sales Service, 1211 Geneva 27, Switzerland. *International Guiding Principles for Biomedical Research Involving Animals* (1985).
5. Council of Europe. Strasbourg, France. *European Convention for the Protection of Vertebrate Animals used for Experimental and Other Purposes* (1985).
6. U S Governmental Principles for the Care and Use of Laboratory Animals. Federal Register 50 (97) 20864-20865 (May 20th 1985).
7. Steering Committee for the Establishment of a National Code for the handling and use of Animals in Research, Education, Diagnosis and Testing of Drugs and Related Substances in South Africa: *Standards for the Housing and Care of Laboratory Animals* (1989) (see Schedule C).

8. International Air Transport Association (IATA): *Live Animal Regulations*, 11th Edition (1984).

SCHEDULE C

9. STANDARDS FOR THE HOUSING AND CARE OF LABORATORY ANIMALS

9.1 Introduction

There is very little objective data on the space requirements for laboratory animals. Consequently no ideal or perfect system for animal caging can be prescribed for universal adoption. The specifications for caging systems are therefore largely based on successful past experience and professional judgement. The minimum space recommendations detailed in this document are based on those recommended by regulatory authorities in the United States of America and Europe. They are proposed as reasonable norms for the South African teaching and research community to adopt and observe.

9.2 Purpose of the Standards

These specifications have been prepared to establish standards for the care of laboratory animals for the design, constructing and management of laboratory animal facilities. In laboratory animal studies, the most reliable results are likely to be obtained by using healthy animals that are uniform in respect of health, husbandry, housing, genetic characteristics, environmental conditions and nutrition.

Where animal facilities do not conform to these standards it is expected that modifications necessary for the well being of the animals will be made without delay. New facilities must comply with these standards. Nothing in the specifications are intended to imply that uniformity is desirable or should be achieved for its own sake.

9.3 Personnel

Persons responsible for laboratory animals should be thoroughly trained in their care and be familiar with the basic requirements of the animals under normal and experimental conditions. They need to be fully aware of the legal and moral responsibilities associated with the use of animals for scientific purposes.

9.4 The Animal Facility

9.4.1 The animal facility should be designed; sited and constructed to provide suitable environment and incorporate facilities to support all the activities carried out in it. Animal facilities should be designed to be self-contained and have physical barriers, which will prevent the entry of wild animals, insects, parasites and pathogens.

9.4.2 Rooms, which are used for holding, breeding, stock and experimental animals should be constructed with walls and floors which are impervious to water.

9.4.3 Animal rooms should be capable of holding caged or penned animals in accordance with the specification detailed in this standard document.

9.4.4 Care and pen stocking rates should not exceed group and individual animal space allocations specified for the various common laboratory species, without special approval being obtained from the institutional regulatory authority (Animal Ethics Committee) for any such deviations.

9.4.5 Species which are either behaviourally incompatible or of a different health status or require differing environmental conditions should not be housed together within the same barrier system.

9.4.6 Animals, which are infected with parasites or pathogens which are transmissible to man or other animals held nearby, should be effectively managed to prevent the spread of disease.

9.4.7 Breeding animals should normally be accommodated apart from animals, which are being used for experimental procedures. Animals of differing microbiological or genetic status should also be separated by physical barriers. The mechanical transmission of pathogens should be controlled by management procedures, which are formally documented and complied with by each animal facility.

9.5 Environmental Considerations

9.5.1Effect of Environment on Experimental Results

Both experimental results and the comfort and welfare of laboratory animals may be influenced by environmental conditions. Fluctuating environmental conditions are likely to cause variability in the biological responses of laboratory animals. To identify and measure an experimental response in animals against a background of environmental variability may require greater numbers of animals to be used. Good control of variables such as light intensity, photo-period ventilation rate, temperature, relative humidity and noise can contribute to both good scientific practice and minimise animal usage.

9.5.2Temperature Specifications

Animal room temperatures should be maintained in a range of 4 °C within the optimal range specified for laboratory animal species as follows:

	Optimal Range in °C
Non-human primates (South African)	15-24
Mouse, rat, hamster, gerbil	19-23
Guinea pig	16-23
Rabbit	16-20
Pigeon	15-24
Domestic fowl and duck	12-24
Cat, dog, pig	15-24
Goat, sheep, cattle, horse	10-24

9.5.3Relative Humidity Specifications

Extreme variations in relative humidity can have adverse affects on the well-being of animals by affecting rate of heat loss, activity and food intake.

The relative humidity in animal rooms should normally be maintained at 55% ± 10% through humidification control, if necessary. This specification should be applied to all species housed indoors.

9.5.4Ventilation Rates

The purpose of ventilation is to:

i) regulate within prescribed limits, temperature and humidity;
ii) reduce the level and spread of odours, noxious gases, dust and infective agents;
iii) provide sufficient air to meet the respiratory needs of the animals.

The ventilation rates of animal rooms should be related to their stocking density and heat generated by animals and equipment which they contain. Ventilation rates for laboratory species are as follows:

	Fresh Air Changes/Hour
Rodents and lagomorphs in fully stocked rooms	15-20
Cats, dogs, non-human primates, goats, pigs and sheep	10-12

Fewer air changes to maintain specified environmental conditions may be acceptable, with lower stocking rates.

The air distribution system and air flow pattern should deliver air to each cage in animal rooms in a proportional manner, whilst avoiding draughts.

The ventilation system should create differential air pressures within animal house barrier zones to minimise the risk of spreading air borne infective agents.

9.5.5Lighting Standards

Animal room lighting should have an intensity 350-400 lux at 1 metre from the floor. Rooms should be evenly illuminated from overhead light sources. The photoperiod should be regulated on a 12 hour light 12 hour dark cycle for the majority of species unless scientific requirements dictate otherwise.

It should be noted that windows which allow fluctuation in light intensity and photoperiod throughout the year, may be beneficial for staff, but have the potential for interfering with temperature control and photoperiod regulation. Windows are a weak point in the maintenance of secure barriers for animal health and general animal house security. They should be omitted to design from rooms in which defined environmental conditions are to be maintained.

9.5.6Noise Control

Excessive noise and vibration and loud and unexpected and unfamiliar sounds can be disruptive and stressful for laboratory animals and noise control should be adopted as a feature of animal management.

9.6 Transportation Of Laboratory Animals

Transport stresses should be minimised by making animals as comfortable as possible in their transport containers. If travel is to be prolonged for more than 12 hours, food and water should be provided.

In meeting animals needs, transportation authority requirements and specifications for transport containers, the standards to be applied for long journeys are to conform with those of the International Air Transport Association as published in their latest edition of the IATA Live Animal Regulation. (see Schedule A).

When the capture, holding in captivity and transportation of non-human primates, other wild animals and reptiles are regulated to the Provincial Nature Conservation Authorities, their regulations in respect of the acquisition of permits for these purposes must be complied with.

At the end of a journey, animals should be removed from their transport containers without delay and inspected prior to their transfer to suitable permanent housing. Sick or injured animals should be examined and treated by a veterinarian.

9.7 Acclimatisation and Quarantine Requirements

Acclimatisation is necessary for animals to recover from the stress imposed by transport and exposure to new accommodation and a new environment. This is a requirement both for animal welfare and for scientific reasons. A documented policy should exist in each animal facility with regard to this requirement as well as for quarantine procedures which will allow the health status of newly arrived animal to be assessed before they come into contact with existing animal populations. Such a policy should be formulated in consultation with either a veterinarian or an environmental physiologist who is knowledgeable about the acclimatisation needs of the common laboratory animal species.

9.8 Care Of Animals

9.8.1Animal Accommodation

Cages and pens comprise one of the most important elements in the physical and special environment of laboratory animals. They should be designed and constructed to facilitate animal well-being, meet research requirements and minimise experimental variables. They should:

i) provide space that is adequate and permits freedom of movement, normal postural adjustments and a resting space appropriate for the species;
ii) provide a comfortable environment;
iii) provide an escape-proof enclosure that confines the animal safely;
iv) provide easy access to food and water;
v) allow adequate ventilation;
vi) meet the biological needs of the animal such as body temperature maintenance, urination, defecation and reproduction when this is required;
vii) avoid the need for unnecessary or harsh methods of physical restraint;
viii) protect the animals from known hazards.

Caging systems should thus facilitate research while maintaining health and comfort of the animals. They should be constructed of sturdy durable materials and be designed to minimise the transmission of infective agents from cage to cage.

To facilitate servicing and cleaning, cages should have smooth impervious surfaces that do not retain dirt. They should be designed with a minimum of ledges, joints and

acute angles, and corners in which bodily wastes, dirt or water may accumulate. The design should allow for the inspection of the cage occupants without disturbing them significantly. Feeding and watering devices should be easily accessible for filling, changes, cleaning and servicing. Cages and pens should be kept in good repair to prevent injury to animals or personnel.

9.8.2Social Environment

Most laboratory animal species are highly sociable and benefit from being housed with companions. They should be given every opportunity for social interaction where non-compatibility or necessary space restrictions may result in aggressive behaviour and injury. Single housing should be provided wherever possible. Any animal which is housed singly should be assessed on a continuous basis to evaluate the effects of isolation on its behaviour and to respond to these in any way which is possible.

Mice, rats, guinea-pigs, rabbits, cats and dogs are sociable animals and where possible should be housed in social groups. Sexually mature animals can, however, become aggressive when housed in single sex groups and professional judgement must be exercised in providing accommodation for such animals. Research requirements may dictate single accommodation arrangements. The need for social interaction and deprivation of this should, however, be consistently borne in mind when such a decision has to be taken.

9.8.3 Minimum Space Specifications for Laboratory Animals Housed in Groups or Singly

Animal space allocations adopted selectively from published national and international standards documents have been used as a basis for these specifications.

SPECIES AND BODY MASS	TYPE OF HOUSING	FLOOR AREA/ANIMAL IN CM^2		CAGE HEIGHT IN CM
		IN GROUPS	SINGLY	
Mice (g)				
10	cage	39	200	12
15-30	cage	78	200	12
Rats and gerbils (g)				
100	cage	110	500	18
100-200	cage	144	500	18
200-300	cage	187	500	18
300=400	cage	258	700	18
400-500	cage	387	700	18

SPECIES AND BODY MASS	TYPE OF HOUSING	FLOOR AREA/ANIMAL IN CM^2		CAGE HEIGHT IN CM
		IN GROUPS	SINGLY	
Hamsters (g)				
60	cage	65	300	15
60-100	cage	103	300	15
over 100	cage	122	300	15
Guinea-pigs (g)				
up to 350	cage	387	900	13
over 350	cage	650	1 000	13
Rabbits (kg)				
2	cage	1 400	2 000	40
2-4	cage	2 500	4 000	40
4-6	cage	3 300	5 000	40
over 6	cage	4 000	6 000	40
Cats (kg)				
3	cage	3 000	5 000	50
over 3	cage	5 000	7 500	50
Dogs (kg)				
15	pen	22 500	45 000	150
15-35	pen	32 500	65 000	200
over 35	pen	40 000	80 000	200
N-H Primates (kg) (Vervet Monkeys and Baboons)				
2-8	cage		3 600	80
8-15	cage	Not	5 600	120
15-30	cage	recommended	8 000	120
over 30	cage		20 000	210
Pigs (kg)				
up to 30		10 000	20 000	100
30-50		13 000	20 000	100
50-100		20 000	30 000	100
100-150		21 000	40 000	100
over 150		37 500	50 000	100
Sheep & Goats (kg)				
up to 35	pen	13 000	20 000	120
over 35	pen	19 000	28 000	120

SPECIES AND BODY MASS	TYPE OF HOUSING	FLOOR AREA/ANIMAL IN CM^2		CAGE HEIGHT IN CM
		IN GROUPS	SINGLY	
Chickens & Ducks (g)				
up to 300	cage/pen	250	350	30
300-600	cage/pen	470	700	40
600-1 200	cage/pen	830	1 250	50
1 200-1 800	cage/pen	950	1 450	50
1 800-2 400	cage/pen	1 200	1 700	55
over 2 400	cage/pen	1 900	2 800	75
Quail (g)				
up to 150	cage/pen	250	350	20
150-250	cage/pen	250	400	25
Pigeons (g)				
800	cage	800	1 225	35

9.8.4Bedding and Nesting Material

Absorbent cage or pen litter and nesting material should be provided unless it is clearly inappropriate. It should be comfortable for the particular species, dry, absorbent, dust free, non-toxic and free from infectious agents, vermin and other forms of contamination.

Sawdust or wood shavings should not be derived from hardwood that has been treated chemically. Nesting materials should provide insulation, and not be harmful to suckling or adult animals. Where large animals are housed on concrete floors either rubber floor mats, grid type floors or pen litter should be used.

9.8.5 Feeding of Laboratory Animals

Diets for laboratory animals should be specially formulated to satisfy the nutritional requirements of each species. They should be properly stored in separate vermin free food stores and be fed before their specified nutritional shelf life expires. Diets should be protected from contamination. The quality of the diet should comply with the dietary Standards for Laboratory Animals Report of the LAC Diets Advisory Committee Lab Anim 11:1-28.

All food hoppers and feeding utensils should be cleaned regularly and maintained in a sanitary condition, especially if moist foods are fed.

Where animals are held in groups, care should be taken to ensure that the non-dominant animals have adequate access to food and water. Obesity in laboratory animals should be prevented by controlling food intake.

9.9 Animal Health Requirements

Most laboratory species are purpose bred and as such are intended to be healthy and free of pathogens and parasites. Healthy animals are a prerequisite for good science and animal welfare. A system for monitoring and maintaining the health

status of laboratory animals should be routinely implemented in an animal facility. Sub-clinical infections may invalidate the data obtained or make the interpretation of results impossible.

It is therefore essential that, in consultation with a veterinarian or other suitable qualified expert on laboratory animal health, a health surveillance programme be adopted and implemented. This should include an effective health system for recording illness and disease. Records of illnesses, diagnosis, treatments, deaths and cause of deaths of animals should be maintained and be available for inspection.

Animals which are intentionally infected with pathogens or parasites should be effectively contained in systems which are appropriate to the hazard posed by the infective agent and, in accordance with regulations specified by the Department of Health and Welfare.

9.10 Pathogen Free Animals

Animals which are stated to be free from specified pathogenic organisms and parasites must be maintained by barrier designated areas in which the air supply and food, water and all items or equipment and supplies which are introduced into the barriered area are adequately sterilised. Personnel working in such units are required to adopt standards of hygiene and to wear clothing which will prevent them from transmitting infections to the animals which are in their care. Regular microbiological surveillance is necessary to ensure that the specified health status of animals is being maintained.

10. LEGISLATION GOVERNING ANIMAL EXPERIMENTATION[2]

In South Africa no separate laws exist to regulate animal experimentation. This is currently under statutory review. Experimental biologists are however subject to legislative control of a wide range of activities which are associated with the use of experimental animals. It is important that a researcher or user of experimental animals adhere to these laws and that the necessary permits or authority be obtained prior to the start of the experiment.

The use of a wide range of wild and domesticated animals for teaching and research is subject to the following laws:

10.1 The Animal Protection Act (No. 71 of 1962)

This Act consolidates all the earlier laws relating to the prevention of cruelty to animals and applies by definition to all domestic and wild mammals, birds and reptiles which are held in the captivity or control of any person. Offences in terms of this Act include cruel treatment, poor housing, malnutrition, neglected parasitism, disease or injury, exposure of animals to danger of attack by other animals and improper conveyance of animals, and are applicable in situations which may arise in the procurement, transportation, housing, breeding and use of experimental animals.

Although the Act was never specifically designed to regulate biological experiments it also defines an offence as the commission or omission of any act which will cause any unnecessary suffering to any animal. The interpretation of the word *unnecessary* is important in this context for whatever may be convenient, desirable or profitable to mankind may not automatically be deemed to be a necessity.

This Act also empowers authorised officers of animal welfare societies without a warrant and at any time with the consent of the occupier, to enter any premises in which any animal is held for the purpose of examining the conditions under which it is kept. If consent is refused by the occupier of the premises, an animal welfare society officer may obtain a warrant from a magistrate which will give him legal authority to enter and carry out an inspection. He/She may also without warrant arrest any person who is suspected on reasonable grounds of having committed an offence under this Act and seize anything in the possession or custody of that person at the time of the arrest and take it forthwith before a magistrate.

There have been several prosecutions of persons who have been found guilty of contravening the Animal Protection Act in the capture, transportation and use of laboratory animals.

10.2 Animal Disease and Parasites Act (No. 13 of 1956)

This legislation consolidates and amends the laws relating to animal diseases and controls the movement of animals from place to place in South Africa and the importation of animals, parasites or infectious agents into the Republic of South Africa. Under this law animals, parasites or infectious agents and animal products specified by the Veterinary Division of the Department of Agricultural Technical Services may only be moved under the authority of a permit issued by a State Veterinarian. These laws are applicable to domestic livestock, pets and indigenous and exotic wild animals.

The laws are complex and any animal users in the University who wish to transport animals, animal products, body fluids or tissues from the field to the University or import exotic animals or special strains of laboratory animals into South Africa should consult the State Veterinarian, State Veterinarian, Allerton Research Laboratories, Pietermaritzburg or 18 Stanger Street, Durban, to establish the procedure which must be adopted to conform with the requirements of this legislation.

10.3 Nature Conservation Ordinance (No. 15 of 1974)

Under this Ordinance the KwaZulu-Natal Provincial Administration established a Nature Conservation Branch for the advancement and control of Nature Conservation. This Ordinance consolidates and amends the laws relating to Wild Animals, Problem Animals, Fisheries and Indigenous Plants and the hunting of game in KwaZulu-Natal.

Under this Ordinance permits are required for:

a) The keeping of wild animals in captivity.
b) The importation into or exportation from KwaZulu-Natal of wild animals.
c) The transportation of wild animals.
d) The importation of exotic animals.
e) The keeping and transportation of problem animals.

Definitions

Wild Animal - any vertebrate animal including birds, reptiles (but not fish) which are kept or bred in captivity or elsewhere, belonging to a non-domestic species whose habitat is either temporarily or permanently in any part of the RSA or Namibia and includes the carcass, egg, flesh (whether fresh or cured), biltong, unprocessed or partly processed hides, skin, thong, tusk, bone, horn, shell, scale, claw, hoof, paw, tail, hair, feather or any other part of such vertebrate animal.

Exotic Animal - any live vertebrate animal including birds, reptiles (but not fish) belonging to a non-domesticated species the habitat of which is not the RSA.

Problem Animal - any species of wild animal or exotic animal alive or dead as contemplated in Subsection 36(1) of the Ordinance. These animals are declared vermin and the Chacma baboon and Vervet monkey are included in this group.

Prohibited Acts in respect of live Problem Animals

Any person who possesses, sells, buys, donates, receives consequent upon a donation, imports, conveys, breeds, releases in the Province, keeps in captivity or controls any live problem animal or is in charge of such an animal without being the holder of a permit issued by the Director of the KwaZulu-Natal Nature Conservation Services whereby he/she is authorised to do so, shall be guilty of an offence.

Animal users wishing to capture animals in the field, collect animal tissues or body fluids or hold wild animals in captivity in University Schools must comply with the requirements of this Ordinance.

This Ordinance is extremely complex and enquiries in respect of its interpretation should be directed to the Head Scientific Services, Ezemvelo KZN Wildlife, P.O. Box 13053, Cascades, 3202.

Similar Nature Conservation Ordinances exist for Gauteng, the Free State and the Cape Provinces. Authority to capture and export animals or animal products will have to be obtained from the relevant provinces.

Veterinary guidelines for the anaesthesia and surgery of free ranging small mammals

Study animals should be captured using methods that cause the least amount of stress and disturbance.

Surgical procedures should be performed as soon as possible after capture and without unnecessary holding and transport stress.

Anaesthesia/immobilisation and surgery must be performed by or under the direct supervision of a suitably qualified Veterinarian. The Veterinarian will ensure that adequate anaesthesia/analgesia is maintained and that surgical procedures are performed in a sterile manner and in accordance with the standards prescribed by the South African Veterinary Association.

Animals should be left to recover in a quiet and dark environment and released as soon as the animal has completely recovered.

The surgical procedure performed must in no way compromise the animal once it has been released back into the wild.

10.4 The Medicines and Related Substances Control Act (No. 101 of 1965)

This Act contains all the laws relating to the regulation and control of medicinal substances. All medicines are classified into one of nine schedules according to the active substances which they contain, in order to control the acquisition and use of these substances. Many of the substances scheduled in this Act are used in animal experimentation.

The substances classified in Schedules 1 and 2 are the so-called Pharmacy Remedies which may be obtained from a pharmacist without a prescription.

Schedule 1 Included in this group are the milder analgesics, the topically applied antibiotics, sulphonamides and local anaesthetics and other injectable substances not classified in the other schedules.

Schedule 2 Contains substances such as the antihistamines, adrenaline, arsenicals, strychnine, alkaloids and glycosides.

Schedule 3 - 7 Substances not directly available to the public and may only be supplied on the prescription of a registered medical, dental or veterinary practitioner. The acquisition of Schedule 3 - 7 substances by persons who are not qualified to write prescriptions for these substances must be through registered veterinarians at e.g. Allerton Regional Veterinary Laboratories, Pietermaritzburg.

Schedule 3 This schedule contains a minor group of prescription drugs such as insulin, digitalis, atropine, thyroid extracts and injectable vitamins.

Schedule 4 This schedule contains the main group of prescription drugs such as the antibiotics, sulphonamides, corticosteroid, diuretics, hormones and local anaesthetics.

Schedule 5 Substances consist of the "minor" tranquillisers and other psychotropic drugs and anaesthetics such as the phenothiazine derivatives, chloral hydrate, halothane, urethane, diazepam, butyrophenones, heparin, and ketamine.

Schedule 6 Contains the "major" tranquillisers and barbiturates and other possible habit forming drugs such as pentobarbitone sodium, and thiopentone sodium.

Schedule 7 Contains the group of therapeutic narcotic substances such as morphine, pethidene, fentanyl, oxymorphone, codeine, opium and etorphine. When stocks of Schedule 7 substances are held, a Schedule 7 register must be kept by the medical, dental or veterinary practitioner who has to order these substances and who is responsible for their safekeeping and issue for use in laboratory animals.

Prohibited Substances

Schedule 8 Heroin, L.S.D., Cannabis, and Phencyclidine.

Schedule 9 Amphetamine and dexamphetamine.

Schedule 8 substances may not be possessed or used by anyone unless so authorised by the Medicines Control Council and Secretary for Health.

Schedule 9 substances may not be acquired by any person other than the Secretary for Health for the purpose of providing medical practitioners with these substances for the treatment of special patients.

This Act is of importance to University staff and students, because a wide range of Schedule 3 - 7 substances are used in animal experimentation. No persons other than medical practitioners, dentists or veterinarians can assume legal responsibility for the use of Schedule 3 - 7 substances. These substances must be used under the supervision of qualified medical, dental and veterinary practitioners or persons with the appropriate licence.

11. THE USE OF LABORATORY ANIMALS[2]

Proper care and humane treatment of animals during their use in teaching and research requires scientific and professional judgement. This implies specific knowledge of the needs of the animals, the requirements of research, and adequate facilities to carry out the experimental procedures. The guidelines in this section outline the general procedures and standards of practice which are to be adopted by animal users.

11.1 Monitoring the Use and Care of Laboratory Animals

An Animal Ethics Committee has been appointed to monitor the use of all living vertebrate animals for teaching and research in the University. Its main functions are to see that animal care and use is in keeping with the institutional policies and accepted scientific practice, and to assist animals users in overcoming difficulties associated with animal experimentation. This then functions to protect the animal, the institution, and the user from abuse or criticism.

11.2 Requirements and Standards

11.2.1 Application for Clearance of an Animal Experiment

No animal experiment may be commenced until it has been authorised by the Animal Ethics Committee. Formal application must be made by all animal users to the Animal Ethics Committee for all experiments to be performed on living vertebrate animals for teaching and research purposes.

This procedure has been adopted in order to:

a) Prevent objectionable activities.
b) Encourage humane practices.
c) Provide for accountability to the public for all animal experiments which are performed in the University.

It is becoming increasingly common that many overseas journals will not accept a paper for publication unless it is accompanied by proof of clearance following ethical review.

Records of all experiments performed on living animals must be entered in a Register to be kept by the School where the experiments are carried out. Entries must be kept up to date and submitted annually to the University Animal Ethics Committee for inspection.

11.2.2 Anaesthesia and Analgesia

The proper use of anaesthetics, analgesics and tranquillisers is necessary for humane and scientific reasons. The use and choice of the most appropriate drug or combination of drugs are matters of judgement for the investigator concerned. Those researchers unfamiliar with the use of these drugs are advised to consult the staff of the Allerton Regional Veterinary Laboratories.

The procurement and use of all anaesthetics and most analgesics are governed by law. Anaesthetics must be administered to animals by competent personnel who are familiar with the agents and their effects on the animals used.

Muscle relaxants or paralysing drugs are not anaesthetics. They must not be used alone for surgical restraint but may be used with drugs known to be effective anaesthetics or analgesics.

All major surgical procedures must be performed under general anaesthesia. Minor surgical procedures may be done under local analgesia or preferably general anaesthesia. Details of all drugs to be used in all animal experiments must be entered and described in the research protocol for submission to the Animal Ethics Committee.

11.2.3 Euthanasia

"Euthanasia" is the term used to describe the process whereby an animal is killed using a recognised, acceptable humane technique and implies a quiet, painless death without fear or anxiety. The most important criterion for accepting a euthanasia method as humane is that it has an initial depressive action on the central nervous system to ensure immediate insensitivity to pain. The word "sacrifice" used by many researchers is incorrect and should be discouraged.

The method adopted will depend upon the nature of the study, the species of the animal, and the number of animals to be killed. It is strongly recommended that tranquillisers be administered to larger species such as domestic animals including dogs and cats, prior to the application of any euthanasia procedure. The methods of euthanasia may be broadly grouped as follows:

a) Physical - stunning with exsanguination, cervical dislocation, electrocution, decapitation, and shooting (decompression, immersion in liquid nitrogen and exposure to microwaves require additional research).

b) Non-inhalant Pharmacological Agents - overdose of barbiturates, chloral hydrate, T-61.

c) Inhalant anaesthetics - overdose of ether, chloroform, halothane, methoxyflurane and nitrous oxide.

d) Non-anaesthetic gases - carbon dioxide and nitrogen.

e) Tranquillising Agents - massive doses required.

f) Curariform Agents, Strychnine and Nicotine Sulfate - these should *not* be used for euthanasia as they do not have a depressing effect upon the central nervous system, but act at neuro-muscular junctions. The animals dies by asphyxiation caused by paralysis of the respiratory muscles. When these compounds are used, the animal may be conscious and thus subjected to excruciating pain until hypoxia of the brain supervenes.

The administration of a fatal overdose of an anaesthetic agent is generally accepted as the most convenient method of euthanasia. Primates, dogs, cats, guinea-pigs, and rabbits are quickly and humanely killed by injecting barbiturate solutions intravenously or intraperitoneally. Small rodents including rats, mice and hamsters can be killed by cervical dislocation, intraperitoneal barbiturates or by the use of ether (combustible!) or carbon dioxide in an uncrowded chamber.

Euthanasia should only be performed by persons competent in the technique.

11.2.4 Surgery

Distress resulting from inappropriate or inadequately performed surgical techniques or post-operative care constitutes "unnecessary pain". Adequate knowledge of topics such as animal physiology, pharmacology, and anatomy is essential for the success of any research programme involving surgical techniques on experimental animals.

Good surgical techniques, appropriate anaesthesia, proper instrumentation, appropriate facilities and competent pre- and post-operative care are all essential to the welfare of the experimental animal and the success of the surgical project.

The following general obligations apply:

a) The responsibility for the animal in each surgical case lies with the person doing the surgery, who in turn should be accountable to the Animal Ethics Committee for his or her adherence to these standards and for demonstrating an acceptable level of expertise.

b) Where supportive treatment is required (analgesics, tranquillisers, fluids, antibiotics, etc.) the surgical investigator must institute suitable treatment and, where indicated, should consult with the Director of Allerton Regional Veterinary Laboratories.

c) When the animal, as a result of the experimental manipulation, is in distress that cannot be relieved, the investigator should be contacted immediately and the animal euthanased.

d) When an investigator or his/her designate is not available or if the animal is experiencing unnecessary suffering, the Head of School or his/her designate is permitted to take immediate action on animals requiring appropriate veterinary treatment or euthanasia.

e) Multiple major survival surgical procedures on a single animal are discouraged. However, under special circumstances they might be permitted with the approval of the Animal Ethics Committee. Cost saving alone is not an adequate reason for performing multiple survival surgical procedures.

Surgery may only be performed in the following facilities:

a) Laboratory facilities may be used for non-sterile terminal procedures.

b) Laboratory facilities may be used for "clean" surgery on small rodents, birds, amphibians and reptiles. "Clean" surgery entails the use of heat or chemically sterilised instruments and surgical materials; cleansing; depilation and disinfection of the skin surrounding the operative site; and scrubbing and disinfection of the operator's hands.

c) All non-terminal surgery on rabbits, dogs, cats, non-human primates and farm animals must be done aseptically in appropriate facilities. Aseptic surgery should only be performed by individuals qualified by training and experience.

11.2.5 Post-operative Recovery and Care

Appropriate facilities and equipment for all aspects of intensive care should be available for post-operative care of animals. the animal should remain in the designated area until it is stable by vital signs and until close observation is no longer necessary. Records of procedures to date and prescribed post-operative treatment should be maintained. The unit should be competently staffed by qualified personnel when patient-occupied.

It is the responsibility of the experimental surgeon to establish the requirements for post-operative care and treatment. Based on these instructions, follow-up treatment and nursing duties may be performed by qualified personnel. Veterinary consultation and intervention should be available during this period.

Items and procedures that must be considered by the surgeon for the post-operative care of his/her patient include:

a) Watering and feeding by hand or stomach tube.
b) Supplemental heating.
c) Respiratory demands.
d) Fluid and drug requirements.
e) Provision for the treatment of post-surgical shock.
f) Post-surgical analgesia.
g) Care of operative wounds.

11.2.6 Housing, Handling and Nutrition

Except in exceptional cases and then with the approval of the Animal Ethics Committee, all experimental animals will be housed and any live experimental procedure will be conducted within designated animal housing facilities.

Animal facilities should be clean, orderly and free from vermin. The physical comfort of the animal should be a prime consideration of all animal users. Physical comfort applied to housing includes factors such as keeping the animals dry, maintaining animals in a state of relative thermal neutrality, providing sufficient space to assume freedom of movement and allow for normal postural adjustments, avoiding unnecessary physical restraint, and avoiding overcrowding.

All experimental animals should have access to food according to their particular physiological requirements. The food should be clean and free of contaminants and be palatable and nutritionally adequate. It should be fed in amounts which will ensure normal growth in immature animals and maintenance of body weight in adults. Animals on restricted feed studies and on food and water deprivation studies must be closely monitored for undue weight loss or distress.

Drinking water should be available to animals at all times, unless contra-indicated by the experimental protocol. Water should be palatable, clean, and free of taint and bacterial contamination.

11.2.7 Physical Restraint[8]

Brief physical restraint of animals for examination, collection of samples, and a variety of other clinical and experimental manipulations can be accomplished manually or with devices such as restraint stocks or squeeze cages. It is important that such devices be suitable in size and design for the animal being held and operated properly to minimise stress and avoid injury to the animal.
Prolonged restraint of any animal, including the chairing of non-human primates, should be avoided unless essential to research objectives. Less restrictive systems, such as the tether system or the pole and collar system, should be used when compatible with research objectives (Wakeley et al., 1974; Byrd, 1979; Bryant, 1980; Anderson and Houghton, 1983; McNamee et al., 1984). The following are important guidelines for the use of restraint equipment:

Animals to be placed in restraint equipment should be conditioned to such equipment prior to initiation of the research.

The period of restraint should be the minimum required to accomplish the research objectives. Prolonged restraint for any reason must be approved by the Committee.
Restraint chairs or similar devices are not to be considered "normal" methods of housing, although they may be required for specific research objectives.

Restraint chairs or similar devices must not be used simply as a convenience to investigators in handling or managing animals. When such devices are used, their use must be specifically approved by the Committee.

Attention must be paid to the possible development of lesions or illnesses associated with restraint, including contusions, decubital ulcers, dependent oedema, and weight loss. If these or other problems occur, veterinary care and treatment must be provided and the animal, if necessary, must be temporarily or permanently removed from the restraint device.

11.2.8 Animal Health

Signs of ill health or disease must be recognised and appropriate steps must be taken to treat the animals and to control the spread of the disease. The staff of the Animal Housing Facility must be informed immediately so that the necessary action may be implemented.

11.2.9 Student Usage of Animals

When animals are used by undergraduate students for their education or for research, such work shall be under the direct supervision of an experienced member of staff of the School in which the project is undertaken. This person will be considered to be primarily responsible for the welfare of animals used by students.

11.2.10 Holiday and Emergency Care

Animal care is a continuous and daily responsibility. Basic animal care must be provided by the staff of the animal facility/ies as an essential service.

Staff must be provided for weekends and holidays and skilled assistance must be obtained in the event of an emergency. The names and telephone numbers of the investigator or his/her nominee must be provided so that technical staff or security personnel may reach the responsible individual after hours every day including

weekends and holidays to ensure that animals will receive attention if any emergency should arise. If the investigator or his/her nominee cannot be contacted, then the qualified staff of the Animal Housing Facility has the authority to take the appropriate action required.

11.2.11 Legal Requirements

As outlined in a previous section, animal users should be aware of the laws applicable to the acquisition, holding and usage of experimental animals and abide by these.

11.2.12 Biological Hazards and Infectious Agents

Wild caught animals (especially non-human primates) and conventional laboratory animals may carry infectious agents which are transmissible to man (zoonoses). Animal users should assess the risk of cross-infection between animals and their staff. Appropriate steps should be taken to inform all personnel associated with exposure about any hazards which may exist. Adequate precautions should be taken to ensure human safety. In infectious disease studies and other situations in which infectious agents are likely to be present in laboratory animals, effective methods of disposal of carcass material and animal wastes must be enforced.

12. SCIENTIFIC REQUIREMENTS IN ANIMAL EXPERIMENTATION[2]

The initial and obligatory stage in planning or designing an experiment which involves the use of animals is the preparation of a protocol. This protocol is a factual, clear and specific document describing the steps which are to be followed in a proposed experiment. The preparation of research and/or teaching protocols forms part of the required procedure for the submission of applications to the Animal Ethics Committee for clearance to perform an experiment on living vertebrate animals.

Success in applying for such clearance depends upon submitting clear evidence of a well prepared and well planned project which is directed towards attaining a clearly stated objective. Judging or expressing opinions on the scientific merit of a research objective are outside the terms of reference of the Animal Ethics Committee. The responsibility for assessing and approving this aspect of a research project lies solely with the applicant's supervisor, the head of the School in which the research project is to be pursued, and the Research Committee. The Animal Ethics Committee has been charged with the responsibility of reviewing all research protocols involving animal experimentation in order to ensure that they have been prepared in accordance with acceptable ethical and scientific standards.

All research projects and teaching activities undertaken at the University of KwaZulu-Natal using animals, other than human subjects, must have prior approval by the University of KwaZulu-Natal Animal Ethics Committee. Should animal work be undertaken at outside facilities/institutions, ethical approval must be obtained from the relevant authority. A copy of the completed application submitted to that authority, together with documentation stating that ethical approval has been granted, must be submitted to the University of KwaZulu-Natal Animal Ethics Committee. Review of protocols is accomplished through the use of the appropriate application form and the Approved Standard Animal Procedures Booklet. The purpose of the application form is to provide information about the proposed use of regulated procedures so that the Animal Ethics Committee can ensure that the use of animals is appropriate, justified and that the likely adverse effects on the animals concerned

can be weighed against the benefit likely to accrue as the result of the programme of work.

Each supervisor (of Hons, Masters or Doctoral students) or research grant holder must submit one application (**"Animal ethics research"**) covering all the animals used in his/her various projects.

Ethical approval for research projects involving observation of animals in their natural habitat is sought by completing the "**Animal ethics field work**" form.

The Head of each School using animals in undergraduate teaching must submit a single application (**"Animal ethics teaching**") in consultation with the relevant module convenor(s) covering all the courses using animals in that School.

Once approved, protocols are renewed on an annual basis. Renewals are done using the relevant short renewal form.

ALL APPLICATION FORMS ARE AVAILABLE FROM THE RESEARCH OFFICE IN ELECTRONIC FORMAT

Before completing the relevant forms this Guide and the *Approved Standard Animal Procedures Booklet* (available from the Research Office) should be studied. Guidelines for the completion of the application forms are provided with each application form.

12.1 Completion of application forms to obtain approval for research protocols using animals

12.1.1 Title of project

The project should be given a short title which contains key words that best describe the project.

12.1.2 Training of staff, research associates, students and technicians authorised to carry out proposed hands-on animal studies

All individuals using animals in research and teaching should be properly trained. The training is provided through a series of lectures and is in three levels. Level 1 is humane care and use of animals and is required of everyone associated with animal research and teaching. Level 2 is presented by species and includes basic handling and common procedures such as injection, bleeding and euthanasia and is needed by anyone with hands-on use of animals. Level 3 is for more specialised procedures such as surgery, containment, primate use, etc. and is needed only if individuals are doing these procedures. Academic staff and their technical support staff (including student demonstrators need Level 1 and will satisfy Level 2 by attending a special multiple species Supervisors/Academic staff Level 2/3 session.

12.1.3 Background Information

A concise account should be given of the present state of relevant knowledge, with key supporting references, if appropriate. Any previous work in the proposed field by the applicant or members of his/her group should be indicated, also with supporting references if appropriate. Details of peer review may be given here.

12.1.4 Statement of Objectives

The problems the applicant wishes to solve during the lifetime of the project should be defined in specific rather than general terms. Longer term aims may be described separately.

12.1.5 Potential Benefits of the Project

A brief statement should be made as to how the experiment is intended to advance biological knowledge in either a fundamental or applied manner.

12.1.6 Plan of work

This section is the most important part of a research proposal. In this section the "hows", "whys" and "whens" of an experiment should be briefly detailed and explained. In describing the plan of work, it is important to show how the individual procedures, with emphasis on the animal procedures, will be used to meet the scientific objectives of the project. Fuller details of the animal procedures are required in a separate section (see below) and need not be set out here. Details must be given of any control groups and statistical treatment of results. This section should establish that the proposed work is well designed scientifically and is likely to meet the objectives of the project.

It should also be shown how individual procedures inter-relate in the sequencing or staging of the project. Flow diagrams may be helpful. A procedure is defined as one or more techniques directed towards a common purpose. Thus the procedure of raising polyclonal antisera includes the techniques used to immunise and sample, all of which should be given by code number in the following section.

In all cases it must be show that non-sentient alternatives are not appropriate for any part of the project and that full consideration has been given to reducing the number of animals used and refining procedures to minimise suffering.

More specific points regarding the selection of animals and experimental design are given below:

12.1.6.1 Selection of an Experimental Animal

Genetic characteristics are among the most important factors to be considered in selecting animals for use in biomedical research.[3] Inbred strains of various species, especially rodents, have been developed to address specific research needs. The homozygosity of these animals is important to ensure the reproducibility and comparability of experimental data.

The need for standardisation in research is essential. However, in the case of living material, standardisation is extremely difficult because living organisms are constantly changing through normal physiological processes such as growth and maturation. In addition these processes are easily influenced by many other factors such as genetic factors, sex, nutrition, infection and environmental factors such as noise, temperature, relative humidity, air quality and ventilation rate, intensity, duration and wavelength of light, as well as the type of housing used and cage stocking density.

The particular species together with other characteristics important to the experiment such as strain, sex, age, weight, source of procurement and any other special characteristics (e.g. adrenalectomised if the animals are purchased thus prepared) should be stated. The animal's housing conditions, diets, and environmental conditions, should therefore also be given in the protocol if these are pertinent to the study.

12.1.6.2 Experimental design

The experimental design should be clearly described in terms of:

i) Arrangements of the experimental animals into groups for various experimental treatments.

ii) The number of animals in the groups should be given and the methods of assigning animals to these groups should be stated (e.g., by random selection).

iii) The selection of parameters to be measured should be given and if possible the statistical method to be used for the analysis of results should be stated.

iv) The methodology to be used for the evaluation of the parameters should be described. References should be included if the methodology has been reported in published scientific literature. If the methodology has been developed during earlier experiments it should be briefly described.

v) All well documented and internationally accepted procedures are detailed in the *Approved Standard Procedures Booklet* and should be referred to by number. Details of procedures not covered in the *Booklet* should be given, using the same format as used in the *Booklet*.

All medicinal substances to be used in the proposed study should be listed together with the name of a medical, dental or veterinary practitioner (if the applicant is not thus qualified) who will assume legal responsibility for supervising the use of these substances.

12.2. Completion of application forms to obtain approval for protocols using animals in teaching

12.2.1 Training of staff, research associates, students and technicians authorised to carry out proposed hands-on animal studies

See Section 11.1.2.

12.2.2 Aims and objectives

A clear and succinct statement should be given of the aims and objectives of using animals in each course. A brief explanation should be given of what the animals are used for in the course and what the students are expected to learn from this aspect of the course. Attention must be given to the skills that the students are expected to acquire. Where the aims and objectives are similar for different courses, these could be grouped together and given a collective set of aims and objectives. The use of animals should be justified, showing clearly how alternatives will not be suitable to meet the aims and objectives of the course. It must be shown that full consideration has been given to reducing the number of animals used and that procedures have been refined to minimise suffering.

12.2.3 Index of procedures

See Sections 11.1.6.1 and 11.1.6.2. Details must be given of the experimental protocols used in the practicals or other learning activities where animals are used. The relevant pages from the practical manuals may be submitted provided they contain sufficient detail (see format of Approved Standard Animal Procedures Booklet). The protocols used in student practicals will then be taken up in the Approved Standard Animal Procedures Booklet for use in subsequent application rounds.

13. REFERENCES

1. The UFAW Handbook on the Care and Management of Laboratory Animals. Sixth edition, edited by T Poole, Longman Scientific and Technical, 1987.
2. The Care and Use of Laboratory Animals: A guide for the University of the Witwatersrand.
3. Ingle D J (1966), Scientific and Ethical Responsibilities associated with the Use and Care of Animals. Federation Proceedings 25 - 1465.
4. UFAW (1938), The Aims and Methods of the Universities Federation for Animal Welfare.
5. Canadian Council on Animal Care: Guide to the Care and Use of Experimental Animals. Vol 1 (1984).
6. Russel W M S, Burch R L.: The Principles of Humane Experimental Technique. Charles C Thomas, Springfield, II. 1959.
7. Rowsell H C, McWilliam A A.: The right animal for the right reason. In: Proc. 1978/79 CALAS Convention - Canadian Association for Laboratory Animal Science, c/o Bioscience Animal Services, M-524 Biological Sciences Building, University of Alberta, Edmonton, Atla, T6G ZE9. pp 211-220.
8. Public Health Service NIH: Guide for the Care and Use of Laboratory Animals. (Revised 1985).

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