1.0 Preamble

The code of practice for the care and use of animals for scientific purposes should be used as guidance for investigators, researchers, faculties/institutions, animal ethics committee and all involved in the care and use of animals for scientific purposes.

2.0 General Principles for the Care and Use of Animals for Scientific Purposes

2.1 General principles

Encapsulated in these principles is the need in scientific research activities to consider:

- The replacement of animals with other methods
- The reduction in the number of animals used; and
- The refinement of techniques used to reduce the impact on animals.

2.2 The UTAR Scientific and Ethical Review Committee

The committee is responsible for oversight and evaluation of the animal care and use program. Its functions include inspection of facilities; evaluation of programs and animal-activity area; submission of reports to responsible institutional officials; review of proposal uses of animals in research, testing, or education (i.e., protocols); and establishment of a mechanism for receipt and review of concerns involving the care and use of animals at the institution.

The committee must meet as often as necessary to fulfil its responsibilities, but it should meet at least once a month.

2.3 Justification & Responsibilities

2.3.1 Scientific research activities involving animals should be approved only when they are essential to:

- obtain and establish significant information relevant to the understanding of humans or animals;
- the maintenance and improvement of human or animal health and welfare;
- the improvement of animal management or production; or
- the achievement of educational objectives

2.3.2 Studies involving animals should be performed only after a decision has been made that they are justified, ratifying the scientific or educational value of the study against the potential effects on the welfare of the animals.

2.3.3 Researchers who use animals for scientific purposes have an obligation to treat them with respect and consider their welfare as an essential factor when planning and conducting studies.

2.3.4 The acquisition, care and use of animals for all scientific purposes in UTAR must be in accord with this Code of Practice, and comply with all procedures set out in the statues and ordinance of UTAR and/or other written laws of the country.

2.3.5 Researchers have direct and ultimate responsibility for all matters relating to the welfare of the animals they use.

2.3.6 Faculties/Institutions involving animals for scientific purposes must refer to **UTAR Scientific and Ethical Review Committee** (herein referred as **SERC**) to ensure conformation with the standards of this Code.

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

2.3.8 Scientific research activities must not commence until written approval has been obtained from the SERC.

2.4 Replacement:

2.4.1 Techniques, which replace or complement the use of animals in scientific research activities must be sought and used wherever possible.

2.5 Reduction:

2.5.1 Research studies must be scientifically and statistically valid, and must use only the minimum number of animals necessary.

2.5.2 The principle of reducing the number of animals used in scientific activities should not be implemented at the expense of the greater suffering of individual animals.

2.5.3 Scientific activities involving the use of animals must not be repeated unnecessarily.

2.6 Refinement:

2.6.1 Selection of animals must be suitable for achieving the purposes of the investigation taking into account their biological characteristics, including behaviour, genetic constitution and nutritional, microbiological and general health status.

2.6.2 Wildlife should be taken from natural habitats only if animals bred in captivity are not available or are unsuitable for the specific scientific purpose.

2.6.3 Researchers must use the best available scientific techniques and be competent in the procedures they perform.

2.6.4 Studies must be designed to avoid pain or distress to animals. If this is not possible, pain or distress must be minimised.

2.6.5 Pain and distress cannot be evaluated easily in animals and therefore researchers must assume that animals experience pain in a manner similar to humans. Decisions regarding the animal's welfare must be based on this assumption unless there is evidence to the contrary.

2.6.6 An animal, which develops signs of pain or distress of a kind and degree not predicted in the proposal, must have the pain or distress alleviated promptly. If severe pain cannot be alleviated promptly, the animals must be killed humanely forthwith. Alleviation of such pain or distress must take precedence over finishing a study.

2.6.7 Scientific activities which may cause pain or distress of a kind and degree for which anaesthesia would normally be used in medical or veterinary practice must be carried out using anaesthesia appropriate to the species and the procedure.

2.6.8 Pain management appropriate to the species, the procedure and the circumstances must be provided.

2.6.9 Analgesic and tranquilliser usage should at least parallel usage in medical or veterinary practice.

2.6.10 When it is not possible to use anaesthetics or analgesics, such as in certain toxicological or animal production studies or in animal models of disease, the end-point of the experiment must be as early as possible to avoid or minimise pain or distress to the animals.

2.6.11 Neuromuscular blocking agents must not be used without appropriate general anaesthesia, except in animals where sensory

awareness has been eliminated. If such agents are used, continuous or frequent intermittent monitoring of paralysed animals is essential to ensure that the depth of anaesthesia is adequate to prevent pain or distress.

2.6.12 Researchers must avoid using death as an experimental end-point whenever possible.

2.6.13 Scientific activities involving the use of animals must be as brief as possible.

2.6.14 Animals must be transported, housed, fed, watered, handled and used under conditions which are appropriate to the care of the species. The welfare of the animals must be a primary consideration in the provision of care which should be based on the behavioural and biological needs of the species.

3.0 Responsibilities of researchers in the care and use of animals

3.1 General

3.1.1 Researchers have direct and ultimate responsibility for all matters related to the welfare of their animals. They must act in accord with all requirements of this Code.

3.1.2 The responsibility of researchers extends over all facets of the care and use of animals in projects approved by the SERC. This responsibility begins when the animal is allocated to the approved project and ends at the time of disposal of the animals.

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

3.1.4 Researchers should consult other experienced scientists, veterinarians, or laboratory animal, livestock or wildlife specialists when necessary.

3.1.5 Before any scientific research activity involving the use of animals begins, researchers must submit a proposal to the SERC which demonstrates that the project will comply with the conditions of this Code and relevant legislation.

3.1.6 Researchers must not begin a scientific research activity involving the use of animals before written SERC approval is obtained, and must adhere to any requirements of the SERC.

3.1.7 Researchers must ensure that satisfactory arrangements are made for contacting them and other responsible persons in the event of emergencies.

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

3.1.9 Researchers must ensure that records of the use and monitoring of animals in scientific research activities are maintained.

3.1.10 Researchers must inform the SERC when an approved project is completed or discontinued.

3.1.11 Researcher should promptly notify the SERC of any unexpected or adverse effects which occur during the period of the approved project and which impact on the welfare of the animals.

3.2 Project Planning

3.2.1 In addition to the information required by the SERC, researcher needs to address the following questions during the planning stages of a project:

a) Is the project justified ethically and scientifically?
b) Can the aims be achieved without using animals?
c) Has the most appropriate species of animals been selected?
d) Are suitable holding facilities and competent staff available?
e) Have all staff been informed of the planned experimental and other procedures?
f) Is the biological status (genetic, nutritional, microbiological, general health) of the animals appropriate?
g) Are the environmental conditions (including caging or pen type, noise, photoperiod, temperature, humidity, ventilation, density of housing and social structures) appropriate?

h) Are the studies designed so that statistically valid results can be obtained or the educational objectives achieved, using the minimum necessary number of animals?
i) If the scientific research activity could cause the animals any pain or distress, what will be done to minimise or avoid this?
j) What arrangements will be made to monitor the animals adequately?
k) Have any of the studies been performed previously? If so, why should they be repeated?
l) Are there any permits that must be obtained for the importation, capture, use, destruction or release of the animals?

3.3 Conduct of studies

General considerations

Animal Environment, Housing and Management

3.3.1 The physical environment and housing of the animals should

- allow for the normal physiologic and behavioural needs of the animals, including urination and defecation, maintenance of body temperature, normal movement and postural adjustments, and, where indicated, reproduction;
- Be species-appropriate social environment;
- make it possible for the animals to remain clean and dry (as consistent with the requirements of the species);
- allow adequate ventilation;
- allow the animals access to food and water and permit easy filling, refilling, changing, servicing, and cleaning of food and water utensils;
- provide a secure environment that does not allow escape of or accidental entrapment of animals or their appendages between opposing surfaces or by structural openings;
- be free of sharp edges or projections that could cause injury to the animals;
- allow observation of the animals with minimal disturbance of them; and
- have adequate security via a perimeter fence or other means

3.3.2 Animals should be cared for by qualified personnel every day, including weekends and holidays, both to safeguard their well-being and to satisfy research requirements. Emergency veterinary care should be available after working hours, on weekends, and on holidays.

Animal Procurement and Transportation

3.3.3 All animals must be acquired lawfully, and the receiving institution should make reasonable attempts to ensure that all transactions involving animal procurement are conducted in a lawful manner.

3.3.4 Training of animals to cooperate with veterinary and investigative personnel and to enter chutes or cages for restraint or transport should be carried out.

Veterinary Medical Care

3.3.5 Disease prevention is an essential component of comprehensive veterinary medical care. Effective preventive-medicine programs enhance the research value of animals by maintaining healthy animals and minimizing non-protocol sources of variation associated with disease and unapparent infection. These programs consist of various combinations of policies, procedures, and practices related to quarantine and stabilization and the separation of animals by species, source, and health status.

3.3.6 All animals should be observed for signs of illness, injury, or abnormal behaviour by a person trained to recognise such signs. Unexpected deaths and signs of illness, distress, or other deviations from normal in animals should be reported promptly to ensure appropriate and timely delivery of veterinary medical care.

3.3.7 Animals that show signs of a contagious disease should be isolated from healthy animals in the colony. If the entire room of animals is known or believed to be exposed to an infectious agent, the group should be kept intact during the process of diagnosis, treatment and control.

3.3.8 Methods of disease prevention, diagnosis, and therapy should be those currently accepted in veterinary practice.

Care and Use of Animals throughout Research Studies

Limiting pain and distress

3.3.9 Pain and distress cannot be evaluated easily in animals, and therefore researchers must assume that animals experience pain in a manner similar to humans. Decisions regarding their welfare in scientific research activities must be based on this assumption unless there is evidence to the contrary.

3.3.10 The researchers must anticipate and take all possible steps to avoid or minimize pain and distress, including:

- choosing the most humane method for the conduct of the study;
- ensuring the technical skills and competence of all persons involved in animal care and use;
- ensuring that animals are adequately monitored for evidence of pain and distress;
- acting promptly to alleviate pain or distress;
- using aesthetic, analgesic and tranquillizing agents appropriate to the species and the scientific or educational aims;
- conducting studies over the shortest time practicable;

  and

- using appropriate methods of euthanasia.

3.3.11 The use of local or general aesthetics, analgesics or tranquillizers must be appropriate to the species, and should at least parallel their use in current medical or veterinary practice.

3.3.12 Scientific research activities which are liable to cause pain of a kind and degree for which aesthesia would normally be used in medical or veterinary practice must be carried out under anaesthesia.

3.3.13 Distress can sometimes be avoided or minimized by non-pharmacological means. Before a study begins, animals should be appropriately conditioned to the study environment and procedures, and be familiar with handlers. During and after experimental procedures, appropriate nursing to minimize pain and distress, and to promote the well-being of the animals, must be provided.

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

3.3.15 If animals develop signs of severe pain or distress despite the precautions outlined above, they must have the pain or distress alleviated promptly or must be killed humanely and without delay. Alleviation of such pain or distress must take precedence over continuing or finishing the study.

Signs of pain or distress

3.3.16 Researchers should be familiar with the normal behaviour of the animal species chosen, be knowledgeable of signs of pain and distress specific to that species, and must monitor their animals for

these signs.

3.3.17 Animals must be monitored to allow detection of deviations from normal behaviour patterns. Such deviations are often the first indications that animals are experiencing pain or distress. Assessments of change in patterns of sleeping, feeding, drinking, grooming, exploratory behaviour, performance in learning or discriminatory tasks, reproduction or social behaviour should be made.

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

Repeated use of animals in scientific and teaching activities

3.3.19 Individual animals must not be used in more than one study either in the same or different projects, without the express approval of the SERC. However appropriate reuse of animals may reduce the total number of animals used in a project, result in better experimental design, reduce distress or avoid pain to other animals.

3.3.20 When approving studies involving the re-use of animals, the SERC must be satisfied that either, (i) none of the procedures cause the animals pain or distress; or (ii) the second and subsequent studies 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) and that the animals have recovered fully from the first study before further procedures are carried out.

Duration of scientific and teaching activities

3.3.21 Scientific research activities, particularly those which involve any pain or distress, should be as brief as practicable. SERC approval must be sought for the continued long-term use of individual animals: The decision to continue must be based on the clinical well-being of the animal and the absence of aversion to the experimental situation.

Handling and restraining animals

3.3.22 Animals must be handled only by person instructed and competent in methods which avoid distress and do not cause injury.

3.3.23 The use of restraint devices is sometimes necessary for the welfare of the animal and the safety of the handler. Restraint devices must be

used to the minimum extent, for the minimum period required to accomplish the purpose of the study and be appropriate for the animal.

3.3.24 General guides for physical restraint:

- Restraint devices are not to be considered normal methods of housing.
- Restraint devices should not be used simply as a convenience in handling or managing animals.
- The period of restraint should be the minimum required to accomplish the research objectives.
- Animals to be placed in restraint devices should be given training to adapt to the equipment and personnel.
- Provision should be made for observation of the animal at appropriate intervals
- Veterinary care should be provided if lesions or illnesses associated with restraint are observed. The presence of lesions, illness, or severe behavioural change often necessitates temporary or permanent removal of the animal from restraint.

3.3.25 Tranquillizers or anaesthetics may aid restraint but may prolong recovery from the procedure. When these agents have been used, recovery of the animals must be monitored.

3.3.26 Periods of prolonged restraint should be avoided. Where animals are in prolonged restraint, consideration must be given to their biological needs, including their behavioural requirements, and they must be monitored regularly by a veterinarian or other qualified person not participating in the project. If any ill effects are shown, the animal must be removed from the restraint, or the method modified.

Completion of projects

3.3.27 Upon completion of the project, animals must be returned promptly to either normal husbandry conditions or, if appropriate and permitted, to their natural habitat, or be killed humanely.

3.3.28 Where practicable investigators should share with other investigators tissue from animals being killed.

Humane killing of animals

3.3.29 When it is necessary to kill an animal, humane procedures must be used. These procedures must avoid distress, be reliable, and produce rapid loss of consciousness without pain until death occurs. The

procedures should also be compatible with the scientific or educational aims.

3.3.30 The procedures must be performed only by persons competent in the methods to be used, or under the direct supervision of a competent person.

3.3.31 Animals should be killed in a quiet, clean environment, and normally away from other animals. There should be no disposal of the carcass until death is established

3.3.32 Dependent neonates of animals being killed must also be killed or provision made for their care.

3.3.33 When fertilized eggs are used, the method of disposal must ensure the death of the embryo.

Autopsy

3.3.34 Autopsy should be performed when animals die unexpectedly.

Additional considerations

3.3.35 Anaesthesia and surgery must be performed by competent staff with appropriate training and experience. Instructors in surgical or anesthetic techniques must be under the direct and constant supervision of such persons.

Surgery

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

3.3.37 The choice and administration of anesthetic, analgesic and tranquillizing agents must be suitable for the species and appropriate for the purpose of the study.

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

3.3.39 When the animal is not to recover from the surgery, it must be unconscious for the whole procedure, either by continuing the administration of the general anaesthetic or by inducing brain death.

3.3.40 When the animal is to recover from the anaesthetic, surgical

procedures must conform to accepted standards in human and veterinary practice. Analgesics and tranquillizers must be used when required and their use should parallel that in current medical and veterinary practice.

Post-operative care

3.3.41 The comfort of animals must be promoted throughout the post-operative period. Attention should be given to warmth, hygiene, fluid and food intake, and control of infection. The use of analgesics and tranquillizers may be needed to minimize post-operative pain or distress. Care should be taken that animals recovering from anaesthesia do not injure themselves by uncoordinated movements, and that conditions are such that they are not disturbed, attacked or killed by other animals in the same enclosure.

3.3.42 Appropriate clinical records must be kept, accessible to all involved in the post operative care of the animal.

3.3.43 Investigators must ensure that adequate monitoring, treatment and care of post- operative animals is provided. They must ensure that they are fully informed of the animals' condition.

3.3.44 The duties of all staff must be clearly defined and ways of dealing with emergencies established.

3.3.45 Any post-operative animal observed to be in a state of severe pain or distress which cannot be alleviated quickly must be killed humanely without delay.

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

Implanted devices

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

Neuromuscular paralysis

3.3.48 Neuromuscular blocking agents must not be used without adequate general anaesthesia or an appropriate surgical procedure which eliminates sensory awareness. Immobilization of an animal solely with a neuromuscular blocking agent is not acceptable. When these agents are used with an anaesthetic, special care must be taken to ensure the maintenance of an adequate plane of anaesthesia. Since criteria such as the character of respiration and

corneal and flexor withdrawal reflexes cannot be used, continuous or frequent intermittent monitoring of physiological variables such as heart rate, blood pressure, pupil size and the electroencephalogram is necessary, together with the effects on these of mild sensory stimuli. Care is required to ensure that drugs used during procedures do not interfere with this monitoring.

Electro immobilization

3.3.49 Electro immobilization must not be used as an alternative to analgesia or anaesthesia. When its use is proposed for the restraint of animals, SERC must carefully evaluate published evidence to assess whether it may cause distress. If so, an alternative restraint method must be used.

Animal models of disease

3.3.50 The scientific validity of animal models of human diseases rests in part on how closely they resemble a particular disease. Thus the attendant pain and distress of the human diseases may also occur in the animal. Special care must be taken in selecting the appropriate species and the investigator must accept responsibility for ensuring that any pain or distress is minimized and that the SERC is informed of the potential effects of the disease on the animals. The use of painful, distressful or lingering death as an end-point in these studies must be avoided wherever possible.

Modifying animal behaviour

3.3.51 Procedures used to modify an animal's behaviour or to induce it to perform specific tasks depends on motivating the animal. The preferred inducement is positive reinforcement, but the inducement may be some form of biological stress. This stress should be as mild as possible. Severe water, food, social or sensory deprivation must not be used. Painful or noxious stimuli must be limited to those which do not distress human beings, and must be used for the minimum time necessary. Behaviour can usually be modified using procedures that involve no more than a physiological stress, e.g. thirst within the range of the normal experience of the species.

Toxicological studies

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

3.3.53 The end-point of such studies must be as early as is compatible

with reliable assessment of toxicity, and must minimize the extent of any pain and distress.

3.3.54 Investigators must not allow scientific activities to proceed to the painful or distressful or lingering death of animals unless no other experimental end-point is feasible and the goals of the study are the prevention, alleviation, treatment or cure of a life-threatening disease or situation in human beings or animals.

3.3.55 When death is essential as the end-point, the study must be designed to result in the deaths of as few animals as possible.

Scientific and teaching activities involving hazards to humans or other animals

3.3.56 Hazards may arise from sources including viruses, bacteria, fungi, parasites, radiation, radioactivity, corrosive substances, toxins, allergens, carcinogens, recombinant DNA, anaesthetic gases and physical injuries.

3.3.57 Any potential pathogenic effects of these hazards when used in studies must be explained as far as possible to all staff. Tests before, during and after the study may be required for staff.

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

3.3.59 Animals being administered infectious organisms should be quarantined as appropriate, taking into account risks to other animals and to people.

3.3.60 The end-point of studies involving hazardous agents should conform to the requirements for toxicological studies.

agents must be appropriate to a 'worst-case' situation.

Animal welfare and animal health research

3.3.62 When studying ways of improving the health or welfare of animals, investigators may need to design studies that replicate the problem such as injury, trauma, nutritional disorder, physical exertion, disease or environmental stress. Thus, the attendant pain or distress may also be replicated. When such studies are necessary, the investigator must ensure that:

- the principal aim of the project is to improve animal welfare or health;
- alternative methods are not possible, such as the use of animals already subjected to the problem;

- all possible steps are taken to minimize any pain or distress; and
- the end-points of studies conform to the requirements for toxicological studies.

Experimental manipulation of animals' genetic material

3.3.63 All work involving the introduction of foreign DNA into mammalian cells or whole animals must be conducted in accord with guidelines issued by the Genetic Manipulation Advisory Committee and the relevant biohazards committee of the institution.

3.3.64 All proposals to manipulate the genetic material of animals, their germ cells or embryos must also be submitted to SERC for approval.

3.3.65 The manipulation of the genetic material of animals has the potential to affect the welfare of the animals and their offspring adversely. Investigators must inform the SERC of the known potential adverse effects on the well-being of the animals.

3.3.66 The clinical status of animals in which the genetic material has been manipulated experimentally must be monitored for unusual or unexpected adverse effects. Investigators must report such effects to the SERC.

Experimental induction of neoplasia

3.3.67 The site for induction of tumours (neoplasia) must be chosen carefully. Subcutaneous, intradermal and flank sites should be chosen when possible. Footpad, brain and eye sites must not be chosen unless there is no alternative.

3.3.68 Investigators must monitor their animals closely for signs of pain or distress, especially sudden changes in body weight.

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

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

3.3.71 In tumour therapy studies, the end-points chosen must be as early as possible, compatible with reliable assessment of the therapy. Weight changes must be monitored closely. Death from the tumour must not be chosen as an experimental end- point.

Lesions of the central nervous system

3.3.72 Anatomical or chemical lesions of the central nervous system have been widely used to study its structure and function in health and disease. These studies demand special consideration when the lesion produces loss or impairment of limb or trunk movements, loss of sensibility to touch, temperature or pain, impairment of the animal's awareness of its surroundings or impairment of appetite or thirst mechanisms. Special animal care, caging, and other facilities may be needed, and the SERC, in approving such studies, has a particular responsibility to ensure that these facilities are available and that the condition of the animals is closely monitored.

Food or Fluid Restriction

3.3.73 When experimental situations require food or fluid restriction, at least minimal quantities of food and fluid should be available to provide for development of young animals and to maintain long-term well-being of all animals.

3.3.74 Studies involving the withholding or severe restriction of food or water should produce no continuing detrimental effect on the animal. In these studies, the fluid balance and/or body weight must be monitored, recorded and maintained within the limits approved by the SERC.

Foetal experimentation

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

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

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

3.3.78 Eggs must be destroyed before hatching, unless hatching is a requirement of the study. The SERC must approve the arrangements made for the hatchlings.

Research on pain mechanisms and the relief of pain

3.3.79 In studies in which unanaesthetised animals are to be subjected to stimuli designed to produce pain, investigators must:

- ensure that these stimuli limit pain at all times to levels comparable to those which do not distress human beings;

- ensure that the animals are exposed to the minimum pain necessary for the purpose of the procedure; and

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

4.0 Recommended methods of euthanasia for common laboratory animals

4.1 Introduction

Euthanasia is a euphemism for humane killing. In laboratory animal science it is applicable whenever:

- An animal has become sick or moribund or is suffering pain which cannot be alleviated

- Animal numbers in the animal house exceed current requirements (to avoid unnecessary wastage it is vital that breeding programs always be tailored to meet only production numbers approved by the SERC)

- It is a requirement of the project itself e.g. when fresh tissues are required for analysis, or

- An animal has come to the end of its breeding or experimental life.

4.2 Principles

Because euthanasia is an unpleasant task to perform at any time, especially when large numbers of animals are involved, SERC have a special responsibility to ensure that investigators consult with the animal house manager well in advance of work commencing about breeding/supply of animals, as well as accommodation and care of animals for the experimental period.

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

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

4.2.3 Animals should be killed in a quiet, clean environment, and normally away from other animals. There should be no disposal of the carcass until death is established.

4.2.4 Dependent neonates of animals being killed must also be killed or provision made for their care.

4.2.5 When fertilised eggs are used, the method of disposal must ensure the death of the embryo.

4.3 Administration

Euthanasia may be carried out using either physical or chemical means. Factors, which influence choice of method, are:

- sensitivity, training and experience of staff
- species, size, and numbers of animals to be dispatched
- cost and availability of agents/equipment required as well as any hazards involved in their use; and
- the reason for euthanasia and the data to be obtained.

4.4 Recommended Methods of Euthanasia for Small Laboratory

Animals Rats, Mice, Guinea Pigs, and Rabbits

Rats and Mice

- For groups of rats and mice a $C0_2$:0_2 (approx 80:20) mix is recommended. Hose in the $C0_2$:0_2 mix into a purpose built Perspex box (or other container), place the animals inside, further fill with the $C0_2$:0_2, seal, and leave for 3-4 minutes before removing cadavers. Where only pure CO_2 (hospital grade) is available this may be used in the same manner (the important consideration is that gas inhaled by animals is a mix of CO_2 and O_2/air, and not an atmosphere of pure CO_2 only). Death can be ensured by following with cervical dislocation.
- Another method for small numbers of mice and young rats is cervical dislocation. Note: For aesthetic reasons cervical dislocation or for that matter decapitation, of adult mice and rats is not recommended without prior sedation (use of CO_2 narcosis, an injectable agent such as Nembutal i.e. sodium pentobarbitone at 150 mg/kg ip, or an inhalant drug agent. Note that stunning is no longer recommended). Any proposal to use cervical decapitation without prior sedation of animals will need to be justified to the SERC.
- Alternatively where animals are already anaesthetised, overdose using a normal anaesthetic solution of barbiturate e.g. Nembutal (sodium pentobarbitone) at 3-5 times the recommended anaesthetic dose rate i.e.

150 mg/kg. It should be administered by the ip (intraperitoneal) or better the ic (intracardiac) route. Death can be ensured by cervical dislocation or by opening the chest to collapse lungs.

- For neonates, the preferred method is to cool pups first by placing on a non-stick surface in the freezer compartment of the refrigerator for several minutes until movement ceases. Ensure death by using cervical dislocation or decapitation i.e. a guillotine. Some claim that cooling first is not necessary.

Guinea pigs

- For individuals or groups of guinea pigs at any age euthanasia can be carried out using C02:02 as described under Rats and Mice. To ensure death open the chest to collapse the lungs before disposal.

- Alternatively where animals are already anaesthetised, overdose with a normal anaesthetic solution of barbiturate e.g. Nembutal (sodium pentobarbitone) at 3-5 times~ the recommended anaesthetic dose rate i.e. 150 mg/kg. It should be administered by the ip (intraperitoneal) or better the ic (intracardiac) route. To ensure death open the chest to collapse the lungs.

- For guinea pigs weighing less than 400 grams euthanasia may also be carried out by cervical dislocation. This should only be undertaken by an experienced person. For those weighing more than 400 grams first render the animal unconscious (using CO_2 narcosis, Nembutal ip, or an inhalant drug agent). Always check that animals are dead before disposal.

Rabbits

- Adult rabbits are best euthanased using a commercially prepared lethal solution of barbiturate i.e. Euthatal or Lethabarb IV, or the anaesthetic preparation Nembutal (sodium pentobarbitone) at 150 mg/kg iv (or ic where the animal is already anaesthetised). Death can be ensured by opening the chest to collapse the lungs before disposal.

- Small animals can also be euthanased by a blow to the back of the head in the region of the atlanto-occipital joint. This must only be carried out by trained/experienced staff and preferably after first rendering the animal unconscious. Death can be ensured by opening the chest.

- Though generally not recommended, it is possible to euthanase rabbits up to 1.5 kg in weight using C02:02 as described under Rats and Mice.

4.5 Recommended Methods for the Euthanasia of Cold Blooded Vertebrates, Birds, and Large Laboratory Animals

Toads and Frogs; Fish and Tadpoles

Toads and Frogs: Using *Trichaine methane sulphonate (MS 222) at 0.4% in water (neutralised* to pH 7.0) with NaOH, place the animal in a shallow layer of the solution and leave for at least 20 minutes until the lower jaw is opened easily. Kill the animal while still anaesthetised by surgically removing the heart, or placing in the freezer. Fish and Tadpoles: As above only using MS *222 up to 0.1 % in water.* Immerse the animal in the solution and while still anaesthetised kill by placing in the freezer.

Birds

Cervical dislocation or crushing of the skull of birds is the preferred method. Alternatively use $C0_2$:0_2, in a container as for rats and mice, or Lethabarb.

Large Animals

Euthanasia in large animals requires special skill. It must be carried out only by trained/experienced staff and, in order to avoid the risk of injury with the help of an assistant.

The preferred method for individual large animals is anaesthetic overdose, e.g. sodium pentobarbitone at 150 mg/kg B.W. An alternative method for cattle, sheep; goats, deer, horses and pigs is the use of a captive bolt gun, provided that it is in good working order and used only by an authorised, experienced staff member. It should be followed by exsanguinations from the jugular vein.

Young pigs can be euthanased by halothane/oxygen anaesthetic delivered by a mask, followed by intracardiac injection of sodium pentobarbitone at approximately three times the recommended anaesthetic dose ie 150 mg/kg.

In each instance euthanasia should be followed by rapid opening up of the pleural cavities of the chest to ensure collapse of both lungs and removal of any risk of the animal recovering. Note that while in the past potassium chloride, magnesium sulphate or air embolism has been used for euthanasia these must only be used in the anaesthetised animal iv (intravenous) or ic (intracardiac).

SUMMARY TABLE OF APPROVED METHODS FOR EUTHANASIA

SPECIES	RECOMMENDED METHODS
Mice, Rats, and Guinea Pigs For mice, rats, or guinea pigs For neonate rats or mice i.e. 'pinkies'	• Euthanase using a (80:20) COz:Oz mix hosed into a pigs purpose built container. This method is highly recommended as humane, cheap and effective. • An alternative method is to use cervical dislocation. Note that for any animal larger than a mouse this should be preceded by gassing with $C0_2$:0_2 or use of inhalant anaesthetic. Stunning is no longer recommended • or where the animal is already anaesthetised overdose with injectable anaesthetic at three times the anaesthetic dose rate e.g. Nembutal (sodium pentobarbitone at 150 mg/kg) ip or better ic, or inhalant anaesthetic. • Cool (for a few minutes only) in freezer compartment of refrigerator and then carry out cervical dislocation or decapitation. **In all cases death can be ensured by following with cervical dislocation before disposal.**
Rabbits	• Give Lethabarb, or injectable anaesthetic overdose of barbiturate (e.g. sodium pentobarbitone at 150 mg/kg) using the iv route (marginal ear vein) or where the animal is already anaesthetised the ic route (intracardiac), or in young animals the ip route. • Rabbits can also be killed by a blow to the' back of the head or, up to 1.5 kg in weight by COz:O2~gassing (as for rats, mice, or guinea pigs). In either case seek help from an experienced animal technician. **Always ensure death by opening the chest wall to collapse the lungs before disposal.**
Birds	• *Small birds chickens:* Carry out cervical dislocation or crush the bird's skull between the finger and thumb. Alternatively use C02:02 or, • *For Larger birds:* A lethal dose of ip (intraperitoneal) sodium pentobarbitone (Nembutal). • *For embryos* i.e. beyond the mid point of gestation, decapitate

Large Animals	• Anaesthetic overdose is the preferred method for euthanasia of large animals. • Alternatively, a captive bolt may be used, followed by *severance of the jugular vessels. This method must only be carried out by an authorised experienced staff member. • Young pigs can be euthanized by halothane/oxygen anaesthetic overdose delivered by mask followed by intracardiac injection of sodium pentobarbitone. *Other methods for ensuring death following euthanasia are: opening the pleural cavities (so that the lungs collapse), using intracardiac injection of air, potassium chloride, or magnesium sulphate or better, intracardiac sodium pentobarbitone (Nembutal) at three times the recommended anaesthetic dose.

5.0 Guidelines for General Anaesthesia of Common Laboratory Animals

5.1 Principles

Anaesthesia means without (an) feeling (aesthesia) and general anaesthetic agents are drugs which, when introduced into the circulation, typically produce a progressive central nervous system depression with loss of sensation.

In line with the requirements of the NHMRC/CSIRO/AAC Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (6 Edition 1997) the use of local or general anaesthetics, analgesics or tranquillisers must be appropriate to the species and should at least parallel their use in current medical or veterinary practice. Anaesthesia and surgery must be performed by competent staff with appropriate training and experience. There must also be adequate monitoring of the depth of anaesthesia and respiratory depression. Adequate monitoring, treatment and care, including analgesia of post-operative animals must be provided and investigators must ensure that they are fully informed of the animals' condition at all times. Alleviation of pain or distress must always take precedence over continuing or finishing work being undertaken. The attention of principal investigators is drawn to Section 3.3 of the NHMRC Code of Practice.

5.2 General Anaesthesia

General anaesthetic agents are either:

(i) **injectable** - administered using a needle and syringe by the intraperitoneal (ip), subcutaneous (sc), intramuscular (im), or intravenous (iv) routes. Agents are classified as barbiturates, narcotics, sedatives - hypnotics (sleep inducing agents), steroids, or dissociative agents

(ii) **inhalants** - administered by the open/semi-open or closed/semi-closed methods. (Where a gas or volatile liquid anaesthetic is used it can present a health hazard for workers. For this

reasons a proper scavenging system must be used.) Inhalant anaesthetics include methoxyfiurane, halothane and isoflurane.

Depending on the agent, anaesthetics are given individually or in combination. Choice is always based on:

- species/strain, weight, sex and age of the animal
- anticipated duration of the procedure and whether it is to be recovery or not
- appropriateness for the procedure in hand including possible interaction with other substances used; and availability of same
- availability of the equipment required to administer agent(s)
- safety for the worker; and
- given the procedure in hand, the agent's reliability to produce a steady-state balance between anaesthesia analgesia, muscle relaxation and optimum physiological function.

Depending on the route of injection, agents generally take about 1-10 minutes to take effect (inhalants are the fastest and easiest to control, followed by iv, ip, im and sc). Anaesthesia lasts for 10-30 minutes or longer depending on the agent used, and wears off in about 10-60 minutes. Inhalation agents can be used for induction and maintenance of anaesthesia or anaesthesia may be induced by an injectable agent and maintained by a gaseous agent. Inhalation agents have the advantage of being rapidly eliminated from the system, mostly via the lung, which allows rapid recovery. They are particularly useful for long procedures and avoid the need for repeated doses of injectable agents which can be cumulative. When used with an appropriate vaporiser and oxygen delivery system, a means of assisted respiration is always available in an emergency. (Refer Appendix I for some recommended anaesthetic choices in the common laboratory animals i.e. rabbits and rodents.)

5.3 Pre and Post-Operative Care of Animals Undergoing Procedures

In addition to the requirement for choosing the right anaesthetic for the procedure, proper consideration must also be given to pre and post-operative care including non pharmacological means.

5.3.1 Pre-operative Care

- Before any procedure is undertaken animals must be checked for health. Note especially any signs of respiratory disease or discharge. The importance of using animals which have been obtained from a healthy, reliable source cannot be over emphasised.

- While it is generally recommended that food intake be reduced or withdrawn before a procedure (to allow for emptying of stomach contents distally and reduction in the risk of asphyxiation by regurgitation) this is not considered necessary/advisable in the case of rabbits and rodents.

- Animal(s) should be transferred (in their cage/s) to a comfortable corner of the research laboratory for a 2-4 hour conditioning period, and observed.

- Just prior to the administration of premedication drug(s) animals should be weighed, and the premedication and anaesthetic drug doses calculated.

- The next step is to administer the pre-medication agent(s) e.g. atropine and a tranquilliser. Rarely used in rats and mice they can be useful in larger laboratory animals such as the guinea pig or rabbit to reduce bronchial secretions and reduce the risk of cardiac depression. It should be remembered, however, that because of the presence of serum atropinase in up to 30% or more of rabbits, response to this drug may be variable (to overcome this problem administer atropine sc every 30 minutes). In general premedication agents help to calm the animal, reduce the calculated anaesthetic dose required, and provide a smooth induction.

- Twenty to thirty minutes following administration of the premedication agent(s) administer the anaesthetic. Where a volatile anaesthetic is to be given use an anaesthetic mask to deliver the agent (in the case of rabbits or larger animals, intubations may be used but only by those expert in the technique).

- At this point, consideration should be given to the administration of pre-emptive analgesia i.e. the administration of an analgesic agent before a surgical intervention. Pre-emptive analgesia is thought to block pain pathways during surgical procedures and reduce pain experienced in the post operative period. Long acting non-steroidal anti-inflammatory agents and or opiates may be used.

5.3.2 Monitoring throughout the anaesthetic

The following items should be monitored throughout an anaesthetic:

- palpebral reflex;

- pedal reflex (this reflex is not particularly reliable in rodents), tail pinch reflex (rats and mice) or ear pinch reflex (rabbits, guinea pigs) as well as general muscle tone;

- body temperature (animals must be kept warm throughout the procedure eg use a thermostatically controlled heating pad, cover small rodents with bubble wrap) respiratory rate;

- heart/pulse rate, tissue colour, capillary bed refill time; and

- if possible also EEG (or EKG) activity, PO2 and blood pressure.

The use of neuromuscular blocking agents must have specific approval from the SERC (see section 3.3.39 of NHMRC Code of Practice). These agents, which induce immobilisation through muscle paralysis, must always be used in conjunction with a general anaesthetic (using a neuromuscular blocking agent alloyed is not acceptable). The neuromuscular blocking agent should not be given until an adequate depth of general anaesthesia has been achieved. Extreme care must be taken to ensure that anaesthesia and loss of consciousness is maintained for As long as the procedure takes and until the neuromuscular blocking agent wears off.

The animal will need to be artificially ventilated and the following monitored closely:

- sudden changes in heart rate or arterial blood pressure with the application of noxious stimuli

- pupil size (this can be confounded by premedication with atropine which dilates 1 the pupil); and

- end tidal PCO2 (should be no greater than 4.7%) and direct arterial $^{P}CO_2$.

5.3.3 Post-operative Care

- Place the animal on its side, head extended, in a clean, dry, warm (30°C) box on shredded paper free of any dangerous objects which could lead to asphyxiation/injury (e.g. sawdust, water containers or other material which might act as a noose) in a quiet warm room away from strong light and in a position where recovery can be monitored. Do not permit animals to become hypothermic.

- Where an endotracheal tube has been used it should be removed very gently as soon as the swallowing reflex returns. Watch for any sign of regurgitation and if necessary reposition the head to a level below that of the rest of the body so that any fluid which has accumulated drains away.

- Restrict access to food and water until full recovery has taken place. Where recovery is prolonged to several hours animals should be turned carefully every 20 minutes or so to avoid fluid congestion in the lungs.

- Provide warm fluid replacement by injection, i.e. Na lactate, Hartmans solution or 0.9% (normal) saline sc or ip. Give approximately 3-5% of the animal's preanesthetic body weight in ml, e.g. 1-1.5 ml for a 30 g mouse.

- Provide post-operative nursing, antibiotics and analgesia as necessary (see Appendix II). Once animals have fully recovered continue to monitor progress for a further 24-48 hours. Observation of surgical wounds is essential as a check on progress of healing.

- Clinical records must be kept and made available to all staff involved. Duties of staff must be clearly defined and ways of dealing with emergencies established. Animals found to be in a severe state of pain or distress which cannot be alleviated promptly must be euthanized immediately.

Further Information can be found in 'Careful How You Hold Me - an insight into caring for laboratory animals' CD ROM University of Melbourne, 1999.

6.0 Occupational Health and Safety

6.1 Introduction

An occupational health and safety program must be part of the overall animal care and use program. The program must be consistent with federal, state, and local regulations and should focus on maintaining a safe and healthy workplace.

6.2 Hazard identification and risk assessment

6.2.1 Researchers who conduct or staff who support research programs that involve hazardous biologic, chemical, or physical agents (including ionizing and non-ionizing radiation) should be qualified to assess dangers associated with the programs and to select safeguards appropriate to the risks.

6.2.2 An effective occupational health and safety program ensures that the risks associated with the experimental use of animals are reduced to acceptable levels.

6.2.3 Potential hazards, such as animal bites, chemical cleaning agents, allergens, and zoonoses that are inherent in or intrinsic to animal use should also be identified and evaluated.

6.2.4 Health and safety specialists with knowledge in appropriate disciplines should be involved in the assessment of risks associated with hazardous activities and the development of procedures to manage such risks.

6.3 Personnel Training

6.3.1 Personnel at risk should be provided with dearly defined procedures for conducting their duties, should understand the hazards involved, and should be proficient in implementing the required safeguards.

6.3.2 Personnel should be trained regarding zoonoses, chemical safety, microbiologic and physical hazards (including those related to radiation and allergies), unusual conditions or agents that might be part of experimental procedures (including the use of genetically engineered animals and the use of human tissue in immunocompromised animals), handling of waste materials, personal hygiene, and other considerations (e.g., precautions to be taken during personnel pregnancy, illness, or decreased immunocompetence) as appropriate to the risk imposed by their workplace.

6.4 Personal hygiene

6.4.1 It is essential that all personnel maintain a high standard of personal cleanliness.

6.4.2 Clothing suitable for use in the animal facility and laboratories in which animals are used should be supplied and laundered by the institution.

6.4.3 A commercial laundering service is acceptable in many situations; however, appropriate arrangements should be made to decontaminate clothing exposed to potential hazards.

6.4.4 Disposable gloves, masks, head covers, coats, coveralls, and shoe covers might be desirable in some circumstances.

6.4.5 Personnel should wash their hands and change clothing as often as necessary to maintain personal hygiene. Outer garments worn in the animal rooms should not be worn outside the animal facility.

6.4.6 Personnel should not be permitted to eat, drink, use tobacco products, or apply cosmetics in animals' rooms.

6.5 Facilities, procedures and monitoring

6.5.1 A high standard of personal cleanliness is essential, facilities and supplies for meeting this obligation should be provided.

6.5.2 Washing and showering facilities appropriate to the program should be available.

6.5.3 Facilities, equipment and procedures should also be designed, selected and developed to provide for ergonomically sound operations that reduce the potential of physical injury to personnel (such as might be caused by the lifting of heavy equipment or animals and the use of repetitive movements).

6.5.4 Safety equipment should be properly maintained and routinely calibrated.

6.5.5 The selection of appropriate animal-housing systems requires professional knowledge and judgement and depends on the nature of the hazards in question, the types of animals used, and the design of the experiments.

6.5.6 Experimental animals should be housed so that potentially contaminated food and bedding, feces, and urine can be handled in a controlled manner. Facilities, equipment and procedures should be provided for appropriate bedding disposal.

6.5.7 Appropriate methods should be used for assessing exposure to potentially hazardous biologic, chemical, and physical agents where the possibility of exceeding permissible exposure limits exists.

6.6 Information on zoonotic diseases for staff who work with animals

6.6.1 General

A zoonotic disease is any disease transmissible between animals and humans. People at risk of contracting a zoonotic disease are those who work with animals (laboratory research workers, veterinarians, farmers, abattoir workers, wool classers, zoo workers, pet shop owners), or those who own animals as pets at home. Tourists travelling through foreign countries may also on occasion be at risk.

University staffs who work with animals should be made aware of possible zoonotic diseases. Note that while allergies (from animal dander or urine) are not zoonotic diseases, they are sufficiently common among animal technicians and investigators for staff to be reminded that should a rash, weeping eyes, or respiratory wheeze develop, they should seek medical advice as soon as possible. People with a history of symptoms associated with a particular species should in any case, try to avoid working with animals as a career as this will only exacerbate their condition.

Common zoonotic diseases transmissible from almost any species, which could be considered of nuisance value only are ringworm, scabies (mites) and bacillary diarrhoea, eg salmonellosis or shigellosis. A more serious problem which could arise from an infected bite wound is a bacterial septicaemia, eg pasteurellosis. Hydatid disease caused by the intermediate stage of the dog tapeworm *Echinococcus granulosus* and visceral larval migrans in children, where permanent brain or eye damage can be caused by the intermediate larval stages of the common dog or cat round worm *(Toxocara canis or Toxocara cati)* are particularly important zoonotic diseases. A pathogen which can cause abortion in pregnant women or mental retardation in the newborn baby is the protozoan organism *Toxoplasma gondii,* an organism commonly shed by cats.

Three important zoonotic diseases which have recently emerged in Australia are Equine morbillivirus (Hendra Virus) from horses, and Lyssa and Menangle virus from bats. Others Information on Zoonotic Diseases for Staff who work with Animals Endorsed UMAEEC Meeting 2/2000 (minor update February 2002) transmissible from laboratory rodents (reported overseas), although uncommon, are lymphocytic choriomeningitis (LCM) and Hanta virus. Similarly rabies, a viral disease usually transmitted by dog bite, Nipah virus, transmissible from pigs by aerosol, and Bovine Spongioencepholopathy (BSE) from beef, although not present in Australia, can be also fatal. Other examples of some zoonotic diseases associated with farm animals are *Chlamydia sp,* Q fever, tuberculosis, brucellosis, leptospirosis, and anthrax, while an example of a zoonotic disease caught from birds, in particular the parrot family, is the respiratory disease chlamydiosis.

For those who work with macaques (old world primates) in any country, there is the risk of tuberculosis. In addition, the risk of herpes B virus and Ebola virus have been reported as a concern for workers overseas.

6.6.2 Instructions to Departments about their Responsibilities to Staff who Work with Animals

It is important to remember that the risk of acquiring any disease, zoonotic or otherwise, will always depend on the following:

- the incidence of the disease in the community;
- Precautionary measures taken to prevent infection, i.e. inoculations (note that immunisation against the non zoonotic disease tetanus should in any case be kept up to date every 5-10 years); the level of hygiene practised;
- the speed with which action is taken to respond to a problem; and
- the availability of effective drugs and treatment.

To ensure that appropriate treatment is given, it is most important that a good history be given to the clinician including details of any animal species involved. Where an individual presents to the clinician with general ill like symptoms, works with animals that has been in contact with animals recently, this should always be communicated even though there may be no particular recollection of a incident involving an animal.

To ensure that staff fully understand their responsibilities with respect to Occupational Health and Safety, departments should ensure that staff are:

- properly informed about the importance of hygiene, wearing appropriate protective clothing, relevant inoculations and the safe handling of animals, materials and equipment. Information in the form of written instructions about the correct way to handle infectious, hazardous, radioactive, carcinogenic, anaesthetic drugs or other substances should, in any case be provided, together with an opportunity to carry out a dummy run before work commences. This is particularly important for the safe operation of equipment, e.g. autoclaves, fume hoods, anaesthetic machines, and recording devices;
- that all staff at the commencement of employment are immunised against tetanus and that they receive a booster every ten years;
- that staff understand the implicit risks of working with animals, particularly in terms of allergy, asthma and zoonotic disease, and that they know what to do in the event of a problem with respect to:
    - **Testing for allergy (blood and skin).** This may be

organised through the Director of the Student Health Service

- **Asthma.** A baseline lung function study will be performed at the commencement of employment for all staff who work with animals and any others in whom it is indicated by reason of their health or employment. This will be followed by an annual check-up where indicated.

- **Zoonoses.** Note that those who work with non human primates will need to have their Mantoux status tested at the commencement of employment, and then again annually. In addition, a 1 ml baseline serum sample (from a 2 ml blood sample) for freezing and storage must be taken from anyone bitten by a non human primate. This should be collected as soon as possible but in any case no later than 24 hours. A second serum sample may need to be taken several weeks later. Information about the risk of *Herpes B* from macaques should be provided. (refer to UMAWC Policy: *Emergency Advice on Herpes B Virus Infection and Diagnosis).*

- **Working with human blood products.** In this instance immunization against hepatitis B is strongly recommended. This can be obtained through the Occupational Health Service. In some instances *hepatitis A* vaccination may be indicated.

6.7 Animal experimentation involving hazards

6.6.1 In specific safeguards for animal experimentation with hazardous agents, careful attention should be given to procedures for animal care and housing, storage and disbursement of the agents, dose preparation and administration, body-fluid and tissue handling, waste and carcass disposal, and personal protection.

6.6.2 Special safety equipment should be used in combination with appropriate management and safe practices.

6.6.3 An oversight process (such as use of a safety committee) should be developed to involve persons who are knowledgeable in the evaluation of hazards and safety issues.

6.6.4 The use of animals in such studies requires special considerations, the procedures and facilities to be

used should undergo review for specific safety concerns.

6.6.5 Formal safety programs should be established to assess the hazards, determine the safeguards needed for their control, ensure that the staffs has the necessary training and skills, and ensure that the facilities are adequate for the safe conduct of the research.

6.6.6 Special facilities and safety equipment are needed to protect the animal-care and investigative staff, other occupants of the facility, the public, animals and the environment from exposure to hazardous biologic, chemical and physical agents used in animal experimentation.

6.6.7 Facilities used for animal experimentation with hazardous agents should be separated from other animal housing and support areas, research and clinical laboratories, and patient-care facilities and should be appropriately identified; and access to them should be limited to authorized personnel. Such facilities should be designed and constructed to facilitate cleaning and maintenance of mechanical systems.

6.6.8 A properly managed and used double corridor facility or barrier entry system is an effective means of reducing cross-contamination.

6.6.9 Floor drains should always contain liquid or be sealed effectively by other means. Automatic trap priming can be provided to ensure that traps remain filled.

6.6.10 Hazardous agents should be contained within the study environment. Control of airflow that minimizes the escape of contaminants is a primary barrier used in the handling and administration of hazardous agents and the performance of necropsies on contaminated animals. Special features of the facility, such as airlock, negative air pressure, air filters and redundant mechanical equipment with automatic switching, are secondary barriers aimed at preventing accidental release of hazards outside the facility and work environment.

6.8 Medical evaluation and preventive medicine for personnel

6.8.1 A health-history evaluation before work assignment is advisable to assess potential risks for individual investigators.

6.8.2 Periodic medical evaluations are advisable for people in some risk categories.

6.8.3 An appropriate immunization schedule should be adopted. It is important to immunize animal-care personnel against tetanus. Pre-exposure immunization should be offered to people at risk of infection or exposure to such agents as rabies or hepatitis B virus.

6.8.4 Vaccination is recommended if research is to be conducted on infectious diseases for which effective vaccines are available.

6.8.5 Nonhuman-primate diseases that are transmissible to humans can be serious hazards. Animal technicians, clinicians, investigators, predoctoral and postdoctoral trainees, research technicians, consultants, maintenance workers, security personnel, and others who have contact with non-human primates or have duties in nonhuman-primate housing areas should be routinely screened for tuberculosis. A procedure should be established for ensuring medical care for bites and scratches.

APPENDIX I

RECOMMENDED ANAESTHETIC AGENTS FOR THE BEGINNER

As a general rule, smaller species have more rapid metabolic rate and require a slightly higher dose than larger species. Remember when calculating dose rates that the weight of the bowel contents in animals with a large caecum, such as the guinea pig or rabbit, can represent up to 20% of the animal's real body weight and so allowance should be made for this. To be quite safe, lower dosages should be administered initially with incremental (i.e. one third of the original calculated dose) top-up doses given every half hour or so to maintain anaesthesia or animals should be maintained on gaseous anaesthesia.

Monitor body temperature and maintain at 37-38°C for rabbits and laboratory rodents. Use a thermostatically controlled heating pad, or cover animals in bubble wrap.

RATS AND MICE		
Premedication Agent		
Injectable Agents	**Dose**	**Comments**
Atropine (Atropine injection, Apex 0.6 mg/ml) Rarely used in rats and mice.	Rat: 0.05 mglkg sc or ip Mouse: 0.04mg/kg sc or ip	Reduces salivary and bronchial secretions. Protects heart from vagal stimulation, bradycardia, and possible cardiac arrest induced by surgery or drugs such as xylazine. Induce anaesthesia with one of the following 10-15 mins later.

RATS AND MICE		
Anaesthetic Choices		
Injectable Agent/s	**Dose/Method**	**Comments**
xylazine HCl* (Rompun, Bayer; Xylapex, Apex; Xylase, Xylazil, Ilium:20 mg/ml) and ketamine HCl (Ketalar Parke Davis; Ketamav, Mavlab; Ketamil, Ilium; Ketamine injection, Parnell; Ketapex, Apex; Ketavet, Delvet: 100 mg/ml)	Rat: 10 mg/kg ip or im Mouse: 10 mg/kg ip Rat: 75-100 mg/kg ip Mouse: 80-100 mg/kg ip	These two agents can be drawn into the same syringe, ie. they do not have to be injected Parnell; independently. NB xylazine is reported to cause tissue necrosis im and so should be administered in conjunction with ketamine. (If a large number of mice need to be anaesthetised draw up 1 part of xylazine: 1 part ketamine: 18 parts sterile saline and inject 0.2 ml ip into each 20 g mouse). Produces variable anaesthesia in rats. Lasts 20-60 mins.
ketamine (as above) medetomidine* (Domitor, Ciba-Geigy)	Rat: 60 mg/kg ip Mouse: 60 mg/kg ip Rat: 0.5 mg/kg ip Mouse: 1.0 mg/kg ip	Produces 20-30 minutes of surgical anaesthesia.
*Both xylazine and medetomidine can be reversed with atipamezole (Antisedan, Ciba-Geigy) 1 mg/kg ip or sc. This helps reverse any drop in blood pressure and respiratory depression but it also removes any relaxation and analgesia provided by these drugs.		
Pentobarbitone 60 mg/ml (Nembutal, Boehringer Ingelheim)	Rat: 40-50 mg/kg ip Mouse: 40-60 mg/kg ip Young: 10-20 mg/kg ip	Medium to long acting barbiturate lasts 30-40 minutes. Considerable variation between individuals. Dilute 1 in 10 (0.6%) solutions to inject.
alphaxalone 9.0 mg/ml /alphadolone 3.0 mg/ml (Saffan/Alfaxan, Jurox) methohexitone Na (Brietal, E1i Lilly)	Rat: 9-12 mg kg iv Mouse: 10-15 mg/kg iv Rat: 50-65 mg/kg ip 10-15 mg/kg iv Mouse: 10 mg/kg iv	Note: The level of anaesthesia is less reliable ip than iv 5-15 mins of anaesthesia
fentanyl citrate 0.315 mg/ml **fluanisone** 10 mg/ml, (Hypnorm, Janssen**) **midazolam** 5.0 mg/ml (Hypnovel, Roche)	Rat: Give 2.7 ml/kg of the following 4 ml mix ip: Hypnorm 1.0 ml Hypnovel 1.0 ml Sterile Water 2.0 ml Mouse: Give a 20 g mouse 0.2 ml (ie 10.0 ml/kg) of the following 1.0 ml mix ip: Hypnorm 1.0 ml Hypnovel 1.0 ml Sterile Water 2.0 ml	**Hypnorm is now difficult and to obtain in Australia. It has to be imported directly from the UK and requires a licence. These drugs provide surgical anaesthesia for about 20 minutes followed by sleep for up to two hours. The fentanyl component of Hypnorm can be reversed by buprenorphine (see Analgesics)

RATS AND MICE		
Anaesthetic Choices		
Inhalant Gaseous Agents	**Dose/Method**	**Comments**
Methoxyilurane (Penthrane, Abbott) Because of its nephrotoxic properties, this is no longer available in Australia for medical or veterinary use. A number of research labs, however, still have small quantities in stock.	Pour several ml onto a wad of cotton wool in the bottom of a large jar. Place over this a perforated metal pouring platform, lower the animal carefully onto the platform, loosely replace jar lid and wait about 40 - 60 secs or until animal is soundly asleep. Remove it and replace lid on jar to prevent further escape of gas into the working environment.	Once the animal has been induced continue to maintain anaesthesia using a mask. A mask can be prepared by 0.5- 1.0 ml of Penthrane onto a little cotton wool placed in the bottom of a wide-mouthed test tube or a very small jar. Invert the test tube or jar onto the bench top when not in use.
$C0_2$:0_2 (80:20) mix	2-3 litres/min in a clear container	For minor procedures, recovery 2-3 mins.
halothane (Halothane, Rhone Meieux; Halothane, VCA) or isofturane (Forthrane, Abbott; Aerrane, Zeneca Pharmaceuticals)	Must be delivered in O2 via a specialised vaporiser. Require scavenging for human safety. Can be used with a mask, endotracheal tube or in a clear container.	Allows rapid induction and recovery. Also O2 source readily to hand if needed for resuscitation.

GUINEA PIGS		
Pre medication		
Injectable Agent	**Dose/Route**	**Comments**
Atropine (Atropine injection, Apex) 0.6 mg/ml	0.05 mg/kg sc	As a pre-med to decrease salivary and bronchial secretions (guinea pigs have relatively narrow airways) and protect heart from vagal stimulation ie bradycardia and possible cardiac arrest. Give diazepam 10-15 mins after atropine to sedate the animal.
diazepam (Pamlin injection, Parnell Laboratories; Valium, Roche)	5 mg/kg ip, im	(Give diazepam in a separate syringe from the atropine). This produces heavy sedation but no analgesia. Good for sedation for minor non-invasive procedures. If used as a premed to injectable anaesthesia, dose should be reduced. After a further 10-15 mins administer one of the following anaesthetic choices.

Guinea pig		
Anaesthetic Choices		
Injectable Agent	**Dose/ Route**	**Comments**
xylazine* (Rompun, Bayer; Xylapex, Apex; Xylase, Parnell; Xylazil, Ilium: 20 mg/ml) and ketamine (Ketalar, Parke Davis; Ketamav, Mavlab; Ketamil, Ilium; Ketamine injection, Parnell; Ketapex, Apex; Ketavet, Delvet: 100 mg/ml)	5 mg/kg ip or sc 40 mg/kg ip or im	Give these drugs independently, or draw up calculated doses in the same syringe and give ip Note: xylaxine can cause tissue necrosis if given im on its own. Intramuscular injections should be given into the large muscle mass at the front of the hind leg. Lasts 30 minutes.
medetomidine* (Domitor, Ciba-Geigy) and ketamine (as above)	0.5 mg/kg ip 40 mg/kg ip	Anaesthesia last 30-40 minutes. *Both xylazine and medetomidine can be reversed with atipamezole at 1 mg/kg sc (Antisedan, Ciba-Geigy). This helps reverse any drop in blood pressure and respiratory depression but also removes the relaxation and analgesia provided by these drugs.
Pentobarbitone 60 mg/ml (Nembutal, Boehringer-Ingelheim)	30-50 mg/kg ip	Medium to long acting barbiturate, lasts 30-40 minutes or longer. Can be associated with high mortality. Better to use 25 mg/kg ip as induction and maintain on gaseous agent.
fentanyl citrate 0.315 mg/ml and **fluanisone** 10 mg/ml, (Hypnorm**, Janssen) **midazolam** 5 mg/ml (Hypnovel,Roche)	8 ml/kg ip of a mixture of 1 part Hypnorm, 1 part midazolam and 2 parts water for injection.	Give separately. Provides surgical anaesthesia for 30- 40 mins. Recovery takes up to 2 hours. The fentanyl component of Hypnorm can be reversed by buprenorphine (see Analgesics) **Hypnorm is now difficult to obtain in Australia ie it is only available by direct importation from the UK and requires a licence.
urethane (Merck)	1500 mg/kg ip or iv	Long duration (about 3 hours), non-recovery only. Solution needs to be made up fresh on the day and can be carcinogenic to the handler.

Inhalant Gaseous Agent	Dose/Route	Comments
methoxyflurane (Penthrane, Abbott)	In container	Wide safety margin, non- irritant
halothane (Halothane, Rhone Meieux; Halothane, VCA)	In O2 using vaporiser recommended by the manufacturer	Can induce hypotension
isoflurane (Forthrane, Abbott; Aerrane, Zeneca Pharmaceuticals)	In O2 using vaporiser recommended by the manufacturer	Can induce hypotension Guinea pigs are difficult to intubate but can be successfully maintained on a face mask.

RABBITS		
Premedication		
Injectable Agent	**Dose/Route**	**Comments**
Atropine (Atropine injection, Apex) 0.6 mg/ml	1.0-3.0 mg/kg im.	As a pre-med to decrease salivary and bronchial secretions. Follow in 10- 15 mins 15 mins with diazepam to sedate the animal.
Diazepam (Pamlin injection, Parnell Laboratories; Valium, Roche)	1.0-2.0 mg/kg im.	Give diazepam in a separate syringe from the atropine. After 10-15 mins administer one of the anaesthetic choices suggested.
Acepromazine 2 mg/ml (ACP, Delvet; Promex, Apex Laboratories)	1 mg/kg sc	Moderate sedation

Anaesthetic Choices		
Injectable Agent	**Dose Rate**	**Comments**
Xylazine (Rompun, Bayer; Xylapex, Apex; Xylase, Parnell; Xylazil, Ilium: 20 mg/ml) and **Ketamine** (Ketalar, Parke Davis; Ketamav, Mavlab; Ketamil, Ilium; Ketamine injection, Parnell; Ketapex, Apex; Ketavet, Delvet: 100 mg/ml)	5 mg/kg im. 35 mg/kg im.	These two agents can be drawn into the same syringe, ie they do not have to be injected independently.
Pentobarbitone 60 mg/ml (Nembutal, Boehringer-Ingelheim)	30-45 mg/kg ip or better iv (marginal ear vein)	Give to effect. Not recommended for recovery because of its reputation for its high mortality rate.
Alphaxalone 9 mg/ml **/alphadolone** 3 mg/ml (Saffan/Alfaxan, Jurox)	6-9 mg/kg iv (ear vein)	Very good and safe
Propofol (Diprivan, Zeneca; Rapinovet, Schering-Plough)	10 mg/kg iv	Produces light anaesthesia for 5-10 minutes or can be used for induction followed by maintenance on gas as for guinea pigs. Intubation is difficult in rabbits and requires practice. A close- fitting face mask can be used to maintain anaesthesia. (Because of the risk of laryngeal spasm, induction using gaseous anaesthesia in rabbits is generally not recommended).
Medetomidine* (Domitor, Ciba-Geigy)	Give im and follow with isoflurane gas.	Anaesthesia for 30-40 minutes. Domitor can be reversed with Atipamezole (Antisedan, Ciba Geigy) 1 mg/kg ip or sc.

APPENDIX II

ANALGESIA

There are few (if any) reasons for not using analgesics in animals undergoing surgery. Analgesia is best given at the time of, or just after, induction of anaesthesia and before any procedure commences. Pre-emptive analgesia greatly reduces recovery time and the need for post operative analgesic administration. There are two main categories of analgesics, non-steroidal anti-inflammatory agents and opiates. They can be used alone or in combination. There are a number of drugs available in each category, each with a different duration of action. Choice of analgesic should be made carefully taking into account the aim of the experiment and the advice of the AEEC. The following table provides a number of suggestions.

Analgesic Drug Alternatives	**RATS**	**MICE**	**GUINEA PIGS**	**RABBITS**
Pethidine (Pethidine injection, Parnell)	10-20 mg/kg im or sc 2-3 hourly	10-20 mg/kg im. or sc 2-3 hourly	10-20 mg/kg sc or im 2-3 hourly	10 mg/kg sc or im 2-3 hourly
Buprenorphine (Temgesic, Reckitt & Colman)	0.01-0.05 mg/kg sc 8-12 hourly	0.05-0.1 mg/kg sc 12 hourly	0.05 mg/kg sc 8-12 hourly	0.01-0.05 mg/kg sc 8- 12 hourly
Carprofen (Rimadyl, Pfizer)	4 mg/kg sc 24 hourly	5 mg/kg sc 24 hourly	4 mg/kg sc 24 hourly	4 mg/kg sc 24 hourly

ANTIBIOTICS

In order to minimise the risk of infection always use aseptic technique and give initial antibiotic dose at the time of surgery.

Antibiotic Alternatives	RATS	MICE	GUINEA PIGS	RABBITS
Ampicillin (Ampicyn,Rhone-Poulenc Rorer; Australia CSL)	10-30 mg/kg sc 8 hourly	10-30 mg/kg sc 8 hourly	Toxic	Toxic
Chloramphenicol (Chloramphenicol 100, Delvet)	30-50 mg/kg sc 12 hourly	30-50 mg/kg sc 12 hourly	30-50 mg/kg sc or im 12 hourly	30-50 mg/kg sc or im 12 hourly
Oxytetracycline (Terramycin 100, Pfizer; Tetravet Hoechst Rousse)	6-10 mg/kg sc or im 12 hourly	6-10 mg/kg sc or im 12 hourly	Toxic	15 mglkg sc or im 24 hourly
enrofloxacin (Baytril, Bayer)	5 mg/kg sc 12 hourly	5 mg/kg sc im 12 hourly	5 mg/kg sc or 12 hourly	10 mg/kg sc or im 12 houry
trimethoprim - sulphadoxine (Tribactral or Tribrissen, Jurox)	30 mg/kg sc 12-24 hourly	30 mg/kg sc 12-24 hourly	30 mg/kg sc 12-24 hourly	30 mg/kg sc 12-24 hourly
doxycycline (Vibravet, Pfizer)	2.5 mg/kg po 12 hourly	2.5 mg/kg po 12 hourly	2.5 mg/kg po 12 hourly	2.5 mg/kg po 12 hourly

OTHER SPECIES

For other species, eg. birds, cats, dogs, etc. or for information about other anaesthetics/ analgesics, refer to NHMRC document and other resources listed under References and contact your department's nominated veterinarian.

For toads and frogs: Use Tricaine methane sulphonate (MS222) at 0.4% in water (neutralised to pH 7.0 with NaOH). Place the animal in a shallow solution until the lower jaw opens easily ie about 10 minutes. Remove and keep moist. Dust surgical wound with cicatrin powder (Wellcome Australia) following and consider giving post operative analgesia eg saturated solution of aspirin in pond water.

For fish and tadpoles: As above but use a lower concentration of MS 222 ie up to 1.0% only.