GENERAL GUIDELINES FOR ANIMAL EXPERIMENTERS ON CARE AND USE OF ANIMALS FOR EXPERIMENTAL PURPOSES

1.0 RESPONSIBILITY

No animal experimentation should be carried out without the approval of the project by the Animal Care and Use Committee.

The investigator has the ultimate responsibility in all matters relating to the welfare of animals under experimentation and should be competent in the procedures to be carried out.

Training programs for investigators and technicians may need to be instituted to improve levels of competence and to acquire new skills.

In the planning stages of investigations it may become apparent that the animals used will suffer pain and stress. A scientific justification of benefit has to be weighed against the discomfort and suffering imposed on the animals. The investigator should also recognize that this responsibility extends over all facets of animal care; including the routine duties of animal technicians. The investigator should be prepared to consult an experienced laboratory animal or livestock scientist whenever necessary.
2.0 DESIGN OF EXPERIMENTS

Before embarking on projects which necessarily involve inflicting discomfort or pain, such as some studies of pain itself or injury or shock, the investigator should carefully consider the scientific justification for undertaking the project.

To ensure the humane use of animals, the following principles should be adhered to:

i. The adoption of techniques and procedures, including in vitro biological systems, which permit the use of the least number of animals.

ii. The adoption of techniques and the use of a species which will provide valid scientific data.

iii. The use of appropriate statistical design.

iv. The reduction of variation with genetic, microbiological and environmental factors by using animals specially bred for experimental purposes.

Some long-term toxicological and biological product testing, cancer research, infectious disease and some field studies, require continuation of the experiment until death of the animals. In such experiments,
consideration should be given to using clinical signs, biochemical or histological changes, rather than the death of the animal, as the end point of the experiment.

Experiments involving the withholding of food and water should be short in duration (less than 12 hrs) and produce no lasting detrimental effect on the animal.

### 3.0 PAIN AND DISTRESS

In practical terms, it is difficult to evaluate pain and other unpleasant sensations, such as discomfort and distress. The investigator should be aware of the signs of distress in animals under experiment and should limit distress to acceptable sounds, abnormal appetite, decline in body weight, impaired reproduction and reduced resistance to disease.

Mammals, and possibly other vertebrates, should be assumed to experience pain in a manner similar to humans, and hence the indications for analgesia and anesthesia should parallel those acceptable in human and veterinary medical practice.

At the beginning of an investigation change of location, change of feed, or the application of experimental procedures may need to be carried out consecutively rather than simultaneously, to allow animals to adjust without periods of excessive stress.
4.0 HANDLING AND RESTRAINING OF LABORATORY ANIMALS

The taming and socialization of animals to suit human requirements is easiest when they are young. It is possible to tame and socialize adult animals, but greater patience is required. Laboratory bred animals are usually quiet when received from the production colony as a result of frequent handling and contact with humans. Subsequent poor handling techniques, however, may lead to frightened or savage behavior.

Animals should be handled only by person instructed and competent in methods which minimize distress and do not cause injury. The use of specific devices to restrain, in animals, is sometimes necessary for both the welfare of the animal and the safety of handler.

When restraint is necessary, it should be for the minimum period to accomplish the purpose of the experiment. Procedures for which prolonged physical restraint is necessary should be demonstrated to have no adverse effects on the animals. It is often necessary to use proper gear and apparatus for easy handling e.g. gavage for oral feed and care of experimental animals. IACUC expects researchers embarking on the use of animal for experiments to be up to date on such needs if necessary.
5.0 ANAESTHESIA AND ANALGESIA

Any surgical procedure which causes pain, distress or injury, apart from brief simple procedures which involve trivial pain, should be performed under general or local anesthesia or analgesia. Investigators need to ensure that the techniques of sedation, anesthesia and analgesia used are appropriate for the particular species of animal used and the techniques are in accordance with current veterinary and human clinical practice.

Continuous monitoring is essential for all anaesthetized animals.

Electro-immobilisation devices should not be used to produce analgesia as there is no evidence that these devices produce analgesia.

If during an experiment there is evidence that animals are experiencing severe pain and distress which cannot be alleviated quickly, the animal should be humanely destroyed without delay, even if the objective of the investigation has not been achieved.

The use of many analgesic and anesthetics is controlled under the Poisons Act and specific legal stipulations govern their purchase, storage and documentation.
6.0 NEUROMUSCULAR PARALYSIS

Neuromuscular blocking agents should not be used without adequate general anesthesia unless the experimental procedures can be demonstrated to be painless or stress-free. Since the evaluation of the depth of anesthesia is complicated in animals paralysed by such agents, parameters such as systemic blood pressure, heart rate, pupil size and the electro-encephalogram, and the effects of nonreceptive stimulation upon them, should be monitored.

7.0 SURGERY

Distress resulting from inappropriate or inadequately performed surgical technique constitutes unnecessary pain.

Surgical procedures should be performed only by qualified persons with the training and experience. Training for the performance of surgical procedures should be under direct and constant supervision of a competent person.

Surgical procedures should be performed in an area which is maintained in a clean condition and which should contain all the equipment necessary to provide for the health and welfare of the animal during surgery.
8.0 POST-OPERATIVE CARE

Animals recovering from anesthesia should be kept under conditions in which they cannot injure themselves by uncoordinated movements. They should not be placed in a cage with other animals, as some species may annoy, attack, or kill anaesthetized members of the group.

During recovery from surgical procedures, skilled care needs to be provided by a trained technician, with attention given to warmth, cleanliness, proper fluid and food intake and control of infection. The use of analgesics may be desirable to minimize post-operative discomfort.

An animal that is observed to be in a state of severe pain or stress, which cannot be alleviated, should be destroyed immediately and humanely.

9.0 IMPLANTED DEVICES

Following an operation in which a monitoring or sampling device has been implanted, or an internal organ fistulated, skilled and specialized attention is required in the care of the animal. Regular observation is essential to determine signs of stress, pain, or infection, which should be treated immediately.
10.0 FETAL EXPERIMENTATION

Where fetal surgery impairs the ability of the neonate to function independently and without pain or distress, the animal should be humanely destroyed before, or immediately following birth.

When surgical procedures are performed on post-implantation fetuses, such fetuses should be assumed to have the same requirements for anesthesia and analgesia as an adult animals.

11.0 NON-SURGICAL EXPERIMENTS

Animals in many projects are not subject to interference but may experience pain or distress. These experiments include toxicological, pathogenetic, behavioral, biological, and therapeutic product studies, and feeding and grazing experiments outside accepted farming practices. Proposals for such experiments should be approved by the Animal Care and Use Committee and any pain or distress minimized. Adequate arrangements should be made for supervision of experiments at remote locations.

12.0 DURATION OF ANIMAL USAGE AND RE-USE OF ANIMALS

No animal used in a procedure entailing pain or distress should be used in a further recovery experiment, unless it has returned to good health and
wellbeing, and only after the specific approval of the Animal Care and Use Committee has been obtained.

13.0 TERMINATION OF EXPERIMENTS AND EUTHANASIA

Once the objectives of the experiment have been achieved, animals should not remain in the experimentation area, but should be returned to normal conditions or destroyed humanely.

Acute experiments involving surgical procedures, which do not require recovery of the animal, should be terminated while the animal remains fully anaesthetized.

The most important criterion of acceptance of a method of euthanasia, is that which has an initial depressive action on the central nervous system to ensure immediate insensitivity to pain.

Euthanasia should be performed by a suitably trained person. The choice of method depends on the species of the animal and the project for which the animal as used.

Physical methods of dislocation of the neck, stunning and guillotining are satisfactory for small laboratory animals and birds, although care should be taken that animals do not recover after stunning.

Inhalation anesthetics should not be used for euthanasia in unventilated animal rooms, and should be handled with care to avoid toxicity to both
human beings and animals in the vicinity. When used correctly, this method is useful for small animals as it has a rapid depressant action on the brain prior to death.

Chemical agents such as barbiturates are effective. They require skills in injection techniques.

### 14.0 USE OF ANIMALS IN TEACHING

In the current setup IACUC do not expect any animals to be used for teaching purposes until when such requirements foreseen in the future based on health related curriculum in UMP. The proposals for the use of animals in teaching is somewhat similar for research whereby should be approved by IACUC and comply with all relevant legislation, with the principles of this Code and with institutional requirements.

In addition, the use of animals for teaching, the following principles are particularly relevant:

i. Animals should not be used when other techniques such as audio-visual aids will achieve the teaching objective satisfactorily.

ii. If animals have to be used, lower orders of life, e.g. bacteria, fungi, protozoa, and insects should have preference over vertebrates.
iii. If animals are to be used, the number should be kept to the minimum.

iv. If animals are handled, manipulated or interfered with in any way by students, there should be close supervision by trained and responsible supervisors.

v. Anesthesia of animals and/or surgical interference should be carried out by students only if it is absolutely essential for training. Close supervision has to be provided by properly trained and qualified supervisors.

vi. Euthanasia of animals should be carried out by trained staff. In the case of surgery carried out under anesthesia by students, euthanasia by anesthetic overdose can be performed by students, under close supervision.

vii. Students should be trained to handle animals humanely.

viii. Legal requirements for registration of persons who use animals for research, diagnosis, and teaching should be compiled with and a copy of the registration certificate must be submitted to the ACUC.
15.0 KEEPING ANIMALS FOR OBSERVATION

Ideally, the care of animals should be in the hands of a trained technician, and the animals housed under properly controlled conditions, such as those available in a well-equipped animal house.

The following principles should apply:

i. Animals should not be maintained on a long-term basis in a classroom without due consideration to problems of disease and allergy that may arise in the students.

ii. Quarters should be provided specifically for housing animals. These quarters and the animals in them should be supervised by a science teacher of technician experienced in animal care.

iii. Regardless of the type of housing used, certain criteria must be met such as the enclosures should have adequate floor space for each animal and be large enough to prevent overcrowding, adequate ventilation and lighting should be provided, and surfaces that are easily cleaned should be used. Temperature and humidity should be appropriate to the type of animal kept, and a suitable form of shelter should be provided.
iv. Food and water should be available at all times, in adequate amounts for maintenance of good health. Food should be palatable and sufficient in quantity and quality to provide a high standard of nutrition for the species concerned.

v. Students and other animal care staff should be trained to handle the animals humanely.

16.0 DISPOSAL OF ANIMALS

Several options are available for disposal of the animals:

i. Return of the animals to the source
   Animals obtained from farms, hatcheries, home, etc., may not be returned to the source due to the high risk of introduction of infectious disease to the source.

ii. Release of the animal(s) to the wild
   Animal(s) obtained from nature should be returned there only on the advice of relevant wildlife authorities. Non-native animals, domesticated and cage-reared vertebrates of any kind should not be released into the wild.

iii. Release of animal(s) to students
The practice of allowing students to take experimental animals either alive or dead home as pets or for any other purpose should be forbidden. It should not be allowed due to the possibility of transmission of zoonotic diseases.

iv.  *Euthanasia*

If euthanasia has to be carried out, an approved humane method should be used by an adult experienced in the technique chosen.

### 17.0 CHOICE AND SUPPLY OF ANIMALS

#### 17.1 Choice Of Animals

It is noteworthy to point out here UMP currently only can accommodate experimentation on small rodents i.e. mice (Types: ICR or BALB or similar ones) or rats (Kyoto Wistar or Sprague Dawley or similar ones). Such species are to be purchased from established suppliers specialized in breeding and keeping animals for scientific research. Where animals are obtained for the laboratory from a variety of sources, trouble frequently occurs as apparently healthy animals can carry disease, which may devastate a susceptible colony and disrupt regular research and experiments where immunosuppression is used. Animals recently introduced into the animal house should not be used in experiments until they have been appropriately quarantined and their health status has been evaluated by the veterinarian.
In general the species to be used should be selected carefully from those available, taking into consideration such factors as space requirements, availability of caging in the animal house, cost of breeding or buying the animal, maintenance costs, existence of baseline data in the literature, availability of a range of genetically defined strains of mice and rats, surgery is more easily performed on large animals, experiments carried out on species normally kept as pets (cats, dogs, horses) may disturb the general public more than those using rodents or farm animals, primates are expensive to buy and difficult to obtain and special primate facilities are required for breeding in large numbers.

Investigators should familiarize themselves with the variety of genetically defined strains, congenic lines, and mutant genes available in the common laboratory species. They should also become familiar with the animal models that are available for the many human diseases which have a genetic component, and with the pathogenic mutant genes or multiple alleles recognized in many biochemical pathways.

17.2 Cost
Experimental animals are expensive to produce and maintain. Over ordering of animals, poor design of experiments in relation to quality and numbers of animals used and continued maintenance of animals after completion of a research project, are a waste of money allocated for research.
18.0 GENETIC QUALITY

18.1 Genetically Defined Animals
In many types of experimental work, the genetic quality of the animals used is of prime importance. This applies particularly in areas such as oncology and immunology, where experiments involve transplanting tissue from one animal to another. In many other fields it is essential (or at least desirable) to use animals with a uniform genetic background as this removes a major source of variability in biological investigations.

Investigators intending to use genetically defined animals should study the international rules of nomenclature. They should be aware that the background strain of animals on which a mutant gene is maintained may play an important role in the experiment.

Previous infections or concurrent subclinical infections can have a dramatic effect on the results of an experiment. The use of specifically bred disease-free animals and in certain circumstances, provision of special animal quarters, can prevent the frustration of failed experiments and save time and money.

18.2 Gnotobiotic Animals
In the current set up, the animal holding laboratory in UMP is not equipped to handle the care of SPF rodents. However for the understanding of researchers it is also possible to work on such rodents
where they are available. SPF animals are the animal of choice for most experimental work. Where investigations are short duration and do not require immunosuppression, no special requirements are necessary for using SPF animals in the experimental animal quarters, although it is advisable to empty completely the animal units, from time to time, to prevent any build-up pathogens. In the case of long-term experiments (more than 6 months), or where immunosuppression is used, special precaution need to be taken to maintain cleanliness as optimum benefit can only be obtained from clean animals. The use of acidified water, sterilized food, protective filters over cages or barrier maintenance should be considered.

18.3 Introduction to Infection
Introduced animals are the major potential source of infection to the animal house, but passenger viruses can be carried in tumour cell lines or other biological materials.

Where it is necessary to inoculate imported materials or possibly infected biological substances into animals, quarantine should be observed.

19.0 SUPPLY OF ANIMALS

19.1 Planning
The cost of breeding (not done currently in UMP) or of buying the necessary animals must be considered during the planning stages of a
project. When the project is of limited duration, or when only a single experiment is contemplated, it would be quicker (and probably less expensive) to buy animals (even from overseas) than to breed them. It takes months to establish a new colony of an inbred line of rats or mice and to build up a breeding stock capable of producing for example, 10 animals of a particular sex and weight, all ready for use at the same time.

19.2 Importing/Exporting laboratory Animals and Biological Products
The import or export of laboratory animals or biological products is subjected to certain requirements for health, quarantine and certification. These requirements are provided in the Animal Ordinance and subject to review. Intending importers or exporters should contact the proper authorities on such matters.

19.3 Moving Animals Inter-state
Under the Malaysia Quarantine law, the Department of veterinary Services, Ministry of Agriculture) can restrict the inter-state entry or movement of animals. Advice should be sought from the local State Veterinary Department or the Department of veterinary Services (Ministry of Agriculture) before animals is ordered from other states. In some circumstances, it is necessary for a Certificate of Health to accompany animals travelling interstate. Under the Malaysia Quarantine law there are strict regulations regarding the entry into the country of biological materials.
20.0 SAFETY AND TRAINING OF PERSONNEL

20.1 General Precautions
All personnel involved in the care and maintenance of animals and in carrying out procedures on animals should be properly trained and/or supervised, so as to minimize the risk of accidental injury of infection.

If during the course of an investigation, members of staff are exposed to the risk of contracting infectious disease, diagnostic surveillance should be initiated and a program of immunization be carried out in accordance with medical advice for those diseases for which vaccines are available.

Certain precautions should be practiced at all times, to minimize risk to the health of personnel.

Precautions:

i. Use of appropriate protective clothing, including gloves, overshoes, overalls, masks. These should not be worn outside animal area.

ii. Maintenance of high standards of personal hygiene e.g. regular hand washing, showers when entering and/or leaving the animal area.

iii. Prohibition of eating, smoking, and drinking in the animal area.

iv. Proper collection and disposal of carcasses and refuse.

20.2 Quarantine
Quarantine regulations are designed to limit the danger of introducing non-endemic diseases to community or to other animal populations to Malaysia. Personnel should familiarize themselves with the disease risks that imported animals may present.

*Physical hazards*

Injuries can arise from poorly designed equipment or can be inflicted by animals. The risk of bites or scratches from animals can be minimized by:

i. handling animals in the correct manner

ii. Providing and using properly designed equipment to restrain animals

iii. Using sedative drugs where appropriate.

**20.3 Infectious and Zoonotic Disease**

Special hazards exist with certain species of animals which are known to harbor disease causing organisms which can be transmitted to man (zoonosis). While zoonotic disease are not usually found in animals held under laboratory conditions, personal hygiene procedures should be such that transmission do not occur. The following precautions may be necessary:

i. Pregnant women should not be permitted to work with animals due to threat of microbial transmission via animal litter.
ii. Personnel working with animal should be screened annually for any animal to man bound diseases. They should take adequate precaution against being bitten by following recommended procedures such as wearing gloves, protection for the eyes and sedation of animals before handling.

iii. Extra care must be taken if spread mainly by inhalation and therefore dead or sick animal should be handled with care.

vi. Several diseases can also be by spread in contact with infected urine as such should be careful to avoid such contact.

Experiments using necropsy procedures on animals infected with highly contagious organisms should be carried out in ventilated safety cabinets in a designated biohazard room using protective clothing including gloves.

Necropsy or waste material for disposal should be sealed in plastic bags, properly labeled and incinerated. The necropsy room should be properly equipped to provide adequate refrigeration, washing, and disinfecting facilities.

Laboratory animals inoculated with material from other diseased animals should be held and handled under conditions using procedures that will minimize any transmission of infection to humans.
20.4 Other Hazards

20.4.1 Allergens
Laboratory staff may develop allergies to the dander, serum, urine, and other tissue products of laboratory animals. In order to minimize risk and problems associated with animal induced allergy, it is advisable to:

i. Require the routine use of protective clothing in animal facilities. These should not be worn outside the facilities.

ii. Use protective gloves when handling tissues

iii. Carry out prolonged procedures on laboratory animals in a ventilated safety cabinet.

iv. Insist that individuals with a history of susceptibility to allergies avoid or minimize contact.

20.4.2 Anesthetic gases
Chronic, long-term exposure to anesthetic gases may be hazardous to health, and laboratories which frequently use them, should discharge waste gases directly to the outside of the building.

Attention is drawn to the hazards associated with exposure of pregnant woman to certain anesthetic gases.

20.4.3 Tumors
Tumors and other biological material, especially those of human origin which may contain potentially infective agents should be regarded as pathogenic and handled accordingly.

20.4.4 Radioactivity and ultra-violet light
Animals inoculated with radioactive substance including radioactive isotopes or which have been implanted with devices emitting radiation have to be housed so that they do not present a danger to either personnel or the environment.

Carcasses and bedding need to be disposed of and cages decontaminated, in compliance with regulations governing the handling and release of radioactive materials into the environment.

The eye and skin are critical areas for ultra-violet (UV) exposure. If UV lights are used staff should be warned of the hazards and provided with ‘wrap-around’ safety glasses. The source of illumination should be appropriately marked.

20.4.5 Administration of recombinant DNA material to animals
Experiments involving *in vitro* preparation and subsequent administration of recombinant DNA molecules to experimental animals should not be performed without prior consultation with the Animal Care and Use Committee. Special handling off experimental animals used for this purpose may be necessary.