Good Practice Guide
for the use of animals in research, testing and teaching
April 2010
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## Contents

1 Introduction 3

2 Purpose of this Guide 4
  2.1 Purpose 4
  2.2 Scope 4
  2.3 Background to the guide 4
  2.4 Comments on the guide 4
  2.5 Definitions of terms and abbreviations used in this guide 5

3 Acquisition of Animals 7
  3.1 Animals collected from their natural habitats 7
  3.2 Animals obtained from other countries 7
  3.3 Transport of animals 8
  3.4 Admission of new animals into holding areas 8

4 Facilities 9
  4.1 General 9
  4.2 Outdoor holding areas 9
  4.3 Indoor housing 9
  4.4 Environmental factors 10
  4.5 Pens, cages and containers and the immediate environment of the animals 10
  4.6 Farm animals (special considerations) 11
  4.7 Enrichment and environmental complexity 12

5 Management of Animals in Breeding and Holding Areas 13
  5.1 Management and personnel 13
    5.1.1 Facility manager 13
    5.1.2 Personnel 14
  5.2 Husbandry procedures 14
    5.2.1 Food and water 14
    5.2.2 Routine husbandry procedures 14
  5.3 Identification of animals 15
  5.4 Disposal of animal carcasses and waste 15

6 Responsibilities of Investigators 16
  6.1 General 16
  6.2 AEC approval 16
  6.3 Planning projects 17
  6.4 Conduct of experiments 18
    6.4.1 Limiting pain and distress 18
    6.4.2 Animal welfare monitoring of pain or distress 19
    6.4.3 Study endpoints 19
    6.4.4 Repeated use of animals in experiments 20
    6.4.5 Duration of experiments 20
    6.4.6 Handling and restraining animals 20
    6.4.7 Completion of projects 20
    6.4.8 Euthanasia 20
<table>
<thead>
<tr>
<th>Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.4.9</td>
</tr>
<tr>
<td>6.4.10</td>
</tr>
<tr>
<td>6.4.11</td>
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<td>6.4.27</td>
</tr>
<tr>
<td>6.4.28</td>
</tr>
<tr>
<td>6.4.29</td>
</tr>
</tbody>
</table>

**7 Responsibilities of Teachers**

7.1 Tertiary institutions
7.2 Secondary and primary schools

**8 Access to Further Information**

Useful websites
Alternatives

**Appendix 1: Pain: Some Concepts and Definitions**

1. Pain
2. Pain and distress
3. Analgesia and anaesthesia
4. Signs of pain
5. Painful procedures
6. NAEAC’s views on pain relief

**Appendix 2: Animal Care/Veterinary Nursing Courses**

**Appendix 3: Animal Welfare Score Sheet**

How to use the Animal Welfare Score Sheet
The welfare of animals in New Zealand is safeguarded by the Animal Welfare Act 1999 (the Act), which requires those in charge of animals to take all reasonable steps to ensure the physical, health and behavioural needs of those animals, according to both good practice and scientific knowledge, further defining those needs as including:

- proper and sufficient food and water;
- adequate shelter;
- opportunity to display normal patterns of behaviour;
- physical handling in a manner which minimises the likelihood of unreasonable or unnecessary pain or distress;
- protection from, and rapid diagnosis of, any significant injury or disease.

It is acknowledged, however, that the nature of the research, testing or teaching may mean that the general obligations under the Act cannot be met, thus recognising that compromised care and some pain or distress to a small number of animals may result in significant benefits to people, other animals or the environment. For this reason, such use of animals is governed by a self-standing set of provisions (Part 6) within the Act, although, within the constraints of any project, all reasonable steps must be taken to ensure that the physical, health and behavioural needs of those animals are met in accordance with both good practice and scientific knowledge, as is stated in section 80(2)(a)(i) of the Act.

Through the criteria that AEC members are required to take into account, the legislation also ensures that consideration is given to the Three Rs (refinement, reduction and replacement) when animals are manipulated, acknowledging the fundamental importance of these concepts to the humane use of animals in science.

The ethical and philosophical implications of the traditional ways animals have been used, particularly in research, testing and teaching, have come under increasing scrutiny. Both the public and the scientific community are concerned that standards of husbandry and care of animals in laboratories should at least parallel those required in other areas.

It is for this reason that such use of animals carries with it significant responsibilities and strict legislative obligations. For instance, Part 6 of the Act allows such activities to be carried out only in cases where there is good reason to believe:

- that the findings of the research or testing or the results of the teaching will enhance the understanding of human beings, animals, or ecosystems; the maintenance or protection of human or animal health or welfare; the management, protection, or control of ecosystems, plants, animals, or native fauna; the production and productivity of animals; or the achievement of educational objectives; and
- that the benefits derived from the use of animals in research, testing, and teaching are not outweighed by the likely harm to the animals.
2.1 Purpose

The purpose of this guide is to promote the humane and responsible use of animals for scientific purposes and to encourage those using and caring for such animals to adopt the highest standard of husbandry and animal care. It aims to set guidelines for what constitutes “good practice” in the management of animals in the research, testing and teaching environment. It is not intended to be an exhaustive guide, and contains a list of publications for reference purposes for more specific and detailed information.

2.2 Scope

The guide encompasses all aspects of the care and use of animals for scientific purposes in medicine, biology, agriculture, veterinary and other animal sciences, industry and teaching. It includes their use in research, teaching, field trials, and product testing.

The guide provides general principles for the care and use of animals, and specifies the responsibilities of investigators and teachers. It also provides guidelines for the humane conduct of experiments, and for the acquisition of animals and their care.

The guide does not cover issues relating to codes of ethical conduct (CECs) or the formation and function of animal ethics committees. For these, institutions and investigators are referred to the Act itself, as well as to the MAF Policy Information Paper 33 (The Use of Animals in Research, Testing and Teaching: Users Guide to Part 6 of the Animal Welfare Act 1999) and the Guide to the Preparation of Codes of Ethical Conduct for information on legislative requirements including legal definitions, the preparation of CECs and the formation of animal ethics committees (AECs).

2.3 Background to the guide

This guide is adapted from NAEAC’s 2002 publication, Good Practice Guide for the Use of Animals in Research, Testing and Teaching, which was produced to accommodate changes in the legislation brought about by the introduction of the Animal Welfare Act 1999. These, together with the first edition (the 1995 Animal Welfare Advisory Committee (AWAC) Code of Recommendations and Minimum Standards for the Care and Use of Animals for Scientific Purposes) have also drawn extensively from the various editions of the Australian code of practice for the care and use of animals for scientific purposes.

This guide was developed by a representative working group established by the National Animal Ethics Advisory Committee (NAEAC) and has taken account of the further revision to the Australian National Health and Medical Research Council (NHMRC) Code of Practice in 2004 (7th edition). Periodic revisions reflect changes in biological science and in community attitudes.

2.4 Comments on the guide

Comments on this guide are invited and should be addressed to:

The Secretary
NAEAC
PO Box 2526
Wellington 6140
2.5 Definitions of terms and abbreviations used in this guide

(The) Act: Animal Welfare Act 1999

Analgesia: The temporary abolition or diminution of pain perception.

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

Animal: The Animal Welfare Act 1999 defines “animal” as:

a. Any live member of the animal kingdom that is:
   i. A mammal; or
   ii. A bird; or
   iii. A reptile; or
   iv. An amphibian; or
   v. A fish (bony or cartilaginous); or
   vi. Any octopus, squid, crab, lobster, or crayfish (including freshwater crayfish); or
   vii. Any other member of the animal kingdom which is declared from time to time by the Governor-General, by Order in Council, to be an animal for the purposes of this Act; and

b. Includes any mammalian fetus, or any avian or reptilian pre-hatched young, that is in the last half of its period of gestation or development; and

c. Includes any marsupial pouch young.

AEC: Animal Ethics Committee.

Approved project: A project which has been formally approved by a properly constituted AEC, on the basis of a written proposal.

Cachexia: Severe generalised weakness, malnutrition and emaciation.

CEC: Code of ethical conduct.

Distress: Acute or chronic response of an animal caused by stimuli that produce observable biological stress as shown by abnormal physiological or behavioural responses.

Embryonated egg: An egg in the last half of incubation.


ERMA: Environmental Risk Management Authority New Zealand.

Euthanasia: The humane termination of life.

Experiment: Any test or trial for a scientific purpose, including any activity to test an hypothesis or demonstrate a known fact.

Fetus: An unborn mammal in the last half of gestation.

GM: Genetically modified.

Genetic modification: The deletion, change or moving of genes within an organism, or the transfer of genes from one organism to another, or the modification of existing genes or the construction of new genes and their incorporation into any organism.


IATA: International Air Transport Association.
**IBSC:** Institutional Biological Safety Committee.

**Investigator:** A person approved by an AEC to be responsible for the conduct of an approved project involving animals.

**Pain:** An awareness of acute or chronic discomfort, occurring in varying degrees of severity, and resulting from injury, disease, or emotional distress as evidenced by biological or behavioural changes or both.

**MAF:** Ministry of Agriculture and Forestry.

**Moribund:** Approaching death; about to die.

**Manipulation:** The Animal Welfare Act 1999 defines “manipulation” in relation to any live animal as meaning interfering with the normal physiological, behavioural or anatomical integrity of the animal by deliberately

a. Subjecting it to a procedure which is unusual or abnormal when compared with that to which animals of that type would be subjected to under normal management or practice and which involves:
   i. Exposing it to any parasite, micro-organism, drug, chemical, biological product, radiation, electrical stimulation, or environmental condition; or
   ii. Enforced activity, restraint, nutrition, or surgical intervention; or

b. Depriving it of usual care; but does not include:
   i. Any therapy or prophylaxis necessary or desirable for the welfare of the animal; or
   ii. The killing of an animal as the end point of research testing or teaching if the animal is killed in such a manner that it does not suffer unreasonable or unnecessary pain or distress; or
   iii. The killing of an animal in order to undertake research, testing or teaching on the dead animal or on prenatal or developmental tissue of the animal if the animal is killed in such a manner that it does not suffer unreasonable or unnecessary pain or distress.

**Project:** A series of related experiments that forms a discrete piece of research.

**Proposal:** A written outline of a research project put forward for consideration by an AEC.

**Scientific purposes:** All those activities performed to acquire, develop or demonstrate knowledge or techniques in any scientific discipline, including activities for the purposes of teaching, research, diagnosis, product testing, and the production of biological products.

**Tranquillisers:** Drugs which are used to treat anxiety or produce sedation.

Throughout this guide:
- “shall” means that there is a statutory requirement;
- “must” denotes a minimum standard;
- “should” and “may” denotes a recommendation.
Animals should be obtained from breeding and supply facilities that maintain conditions consistent with the Guide or relevant industry code.

### 3.1 Animals collected from their natural habitats

Most species of indigenous fauna are protected by law. The Department of Conservation must be consulted when these species are required. Permits are usually necessary to collect, keep, release or kill protected fauna, and further permits are usually required to import or export such species. Any conditions imposed on permits must be observed.

An animal that is a member of an endangered species must not be used unless the research will be of direct benefit to the conservation of that species or a closely related species and will not further endanger the species.

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

While the definition of a manipulation specifically excludes, in s3 (2) (d) of the Act, the hunting or killing of any animal in a wild state by a method that is not an experimental method, capture and restraint is clearly stressful to varying degrees depending on the species, physiological state and experience of contact with humans. Strategies should be employed to minimise distress during capture and disruption of the colonies from which they are taken. There should be careful choice of suitable capture techniques, skilled persons should be used, and appropriate and safe enclosures or caging should be provided after capture.

Animals must be monitored for signs of distress following capture and appropriate measures taken to minimise the stress and treat any capture-induced trauma.

Fish may be caught using commercial harvesting practices.

The use of live traps must conform to the requirements of section 36 of the Act, which states that such traps must be inspected within 12 hours after sunrise every day beginning on the day immediately after the day on which the trap was set, and any living animal removed. Trapped animals should be protected from predators and environmental extremes, and food and water must be provided as necessary.

### 3.2 Animals obtained from other countries

Under the Biosecurity Act 1993, the exit and entry of animals or animal tissues can be restricted.

Permits must be obtained from MAF for the importation of live animals and their genetic material.

The IBSC must be consulted when genetically modified animals are to be imported and approvals obtained from ERMA through the institutional committee. The housing and use of GM organisms and animals requires specialised transitional and containment facilities for vertebrate laboratory animals. MAF approval must first be obtained for the establishment of transitional/containment facilities. This approval requires an on-site inspection by MAF supervisors.

Permits must be obtained from MAF for the importation of specimens from dead animals.

Permits must be obtained from the Department of Conservation for the import/export of both live and dead specimens of all native New Zealand fauna and animals or plants subject to the Trade in Endangered Species Act 1989 or regulations.
3.3 Transport of animals

Transportation can cause distress due to confinement, movement, noise and changes in the environment and personnel. The extent of any distress will depend on the animals’ health, temperament, species, age, sex, the number travelling together and their social relationships, the period without food or water, the duration, the mode of transport, environmental conditions (particularly extremes of temperature) and the care given during the journey.

The Act (s 22) requires that the person in charge of any vehicle or aircraft, and the master, or where there is no master the person-in-charge, of every ship, shall ensure that the welfare of any animal conveyed therein is attended to, and that the animal is provided with reasonably comfortable and secure accommodation.

The consignor is responsible for arranging for the supply of food and water en route.

Under s23 of the Act, it is an offence to confine or transport an animal in a manner or position that causes the animal unreasonable or unnecessary pain or distress, or to transport it if its condition or health is such as to render it unfit to be transported.

Investigators are referred to the Code of Recommendations and Minimum Standards for the Transport of Animals Within New Zealand or its replacement code of welfare for further detailed requirements associated with transporting animals. Transport by air must be in accordance with IATA regulations.

Animals must be transported under conditions which are appropriate to the species and which meet standards generally adopted in veterinary and laboratory animal medicine to ensure that the welfare of the animals is not unduly compromised. Potential sources of distress should be identified and steps taken to avoid or minimise their effects on the animals.

Containers must be escape and tamper proof, provide an environment appropriate to the species and protect animals from sudden movements and extremes of climate.

Food and water should be provided when necessary.

Both the suppliers and recipients of animals must ensure that delivery procedures are satisfactory. Suppliers should notify recipients of transport details (i.e. flight number and estimated time of arrival) to ensure swift delivery. Recipients should ensure that animals are received by a responsible person and transferred to holding accommodation without delay.

The transfer of GM animals between approved institutional containment facilities requires approval from the regional MAF supervisor prior to shipment.

3.4 Admission of new animals into holding areas

When new animals are being admitted into animal holding areas, they should be quarantined and inspected by a qualified person. Their health should be evaluated, treatment instigated if required, and their suitability for the proposed experiments assessed. This period should also allow their acclimatisation to the holding facility and personnel.

The duration of quarantine varies for imported animals. Animals must be housed physically separated from other animals and in general no experimental manipulations are permitted while animals are held in quarantine. However, animals may be bred while in quarantine.

Animals which do not adapt satisfactorily to their new environment should not be kept.
4. General

Facilities include the buildings, tanks, yards or paddocks in which animals are kept. Investigators, AECs and the institutions must ensure that facilities are appropriately designed, constructed, equipped, staffed and maintained to ensure the health and welfare of the animals and to fulfil scientific requirements.

The design and management of facilities will depend on the type of animals to be kept and the experiments to be undertaken. The overall condition and management of facilities should permit effective maintenance and servicing and be compatible with maintaining good health and welfare.

4.2 Outdoor holding areas

These should be compatible with the needs of the species, provide adequate shelter and water, protect the animals from predation, meet other species-specific needs and should comply with established farm or zoological garden practice.

4.3 Indoor housing

Buildings should be compatible with the needs of the animals to be housed and the projects undertaken. Facilities for free movement and group contact are especially important for some species of animals.

Buildings should be designed and operated to control environmental factors appropriately; to exclude vermin and to limit contamination associated with the keeping of animals; to allow the delivery of food, water and bedding; and facilitate the entry of people and other animals.

Buildings should be maintained in good repair. Walls and floors should be constructed of durable materials that can be cleaned and disinfected readily.

Buildings should be kept clean and tidy, and operated to achieve maximum possible hygiene. A pest control programme should be run to monitor and control vermin.

There should be adequate storage areas for food, bedding and equipment. Food should be stored in a way which minimises deterioration of nutritional value and palatability and prevents contamination by feral rodents and other pests.

Detergents, disinfectants and pesticides may contaminate the animal’s environment and choice of agents should be made in consultation with investigators. Deodorants designed to mask animal odours should not be used in animal housing facilities, as they are not an acceptable substitute for appropriate cage and equipment cleaning practices, nor for adequate ventilation. Furthermore, they expose animals to volatile compounds which can alter metabolic processes.

Frequency and intensity of cleaning and disinfection needs to provide a healthy environment, in accord with the animal’s normal behaviour and physiology. Cleaning practices should be monitored on a regular basis to ensure effective sanitation; this can include visual inspection, monitoring water temperatures and microbiological testing of surfaces after cleaning.

There should be a reticulated water supply and proper facilities for drainage, if appropriate.

There should be adequate contingency plans to cover such emergencies as flooding and fire, or the breakdown of lighting, heating, cooling or ventilation.
In the interest of disease prevention and general animal welfare, access to the animal quarters by unauthorised persons should be avoided.

4.4 Environmental factors

Animals should be provided with environmental conditions which suit their behavioural and biological needs unless otherwise approved by the AEC for the purposes of a project.

Air exchange, temperature, humidity, noise, light intensity and light cycles should be maintained within limits compatible with the health and well-being of the animals.

Effective ventilation is essential for the comfort of animals and the control of temperature, humidity and odours. Ventilation systems should distribute air uniformly and achieve adequate air exchange, both within cages and within a room.

Noxious odours, particularly ammonia, should be kept to a level compatible with the health and comfort of the animals and personnel. The adequacy of the ventilation system, the design, construction and placement of cages and containers, population densities both within cages and within a room, the effectiveness of the cleaning and the frequency of bedding changes will all influence the level of noxious gases. Attention should be given to the balance between the need for cleanliness and the potential impact of cleaning procedures on the animals.

Environmental factors potentially affect the welfare of the animals and may affect the results of experiments. Investigators should be consulted prior to any planned changes to the environmental conditions of their animals.

4.5 Pens, cages and containers and the immediate environment of the animals

Animal accommodation should be designed and managed to meet species-specific needs. Pens, cages and containers should be designed, constructed and maintained to ensure the comfort and well-being of the animals. Any variations to these requirements as part of a project must receive prior AEC approval. The following factors should be taken into account:

- species-specific behavioural requirements, including the availability and design of space to allow free movement and activity, sleeping, enclosed spaces, contact with others of the same species, and the opportunity to perform a species-specific behavioural repertoire;
- species-specific environmental requirements such as lighting, temperature, air quality, appropriate day/night cycles and protection from excessive noise and vibrations;
- provision of single housing for animals when appropriate for the species and if necessary for the purpose of the experiment, e.g. during recovery from surgery or collection of samples;
- the need to provide ready access to food and water;
- the need to clean the pen, cage or container;
- protection from spread of pests and disease;
- requirements of the project; and
- the need to observe the animals readily.

Pens, cages and containers should:

- be constructed of durable, impervious materials;
- be kept clean;
- be maintained in good repair;
- be secure and escape-proof;
- protect the animals from climatic extremes;
- not cause injury to the animals;
- be large enough to ensure the well-being of the animal or animals, with adequate space to allow them to stretch out when recumbent and to stand upright.

Wire floor cages for rodents should not be used unless essential to the research protocol and then only for brief periods. Animals should have a solid resting area when housed on wire floors.

The population density of animals within cages, pens or containers and the placement of these in rooms should be such that acceptable social and environmental conditions for the species can be maintained. Where it is necessary to individually house animals of a species which is normally kept in a social group, the conditions should be managed so as to minimise the impact of social isolation. Animals should be housed in these circumstances for the minimum time necessary.

Bedding, litter or other environmental provisions should be provided if appropriate to the species, and should be comfortable, absorbent, dust-free, non-palatable, non-toxic, able to be sterilised if needed and suitable for the particular research purpose. Pregnant animals must be provided with nesting materials where appropriate.

Investigators and animal carers are referred to the publication *Housing for Laboratory Rats, Mice, Guinea Pigs and Rabbits* by A.L. Hargreaves published by ANZCCART in 2000 for more detailed information.

The AEC and relevant investigators or teachers should be informed in advance of planned changes to these conditions since these may affect the welfare of the animals and the results of experiments.

### 4.6 Farm animals (special considerations)

The factors, criteria, and considerations for caging and housing discussed previously also apply to farm animals. However, animals on farms may be housed under less stringent conditions.

In general, housing and management practices should be designed to provide optimal animal care, and should follow the standards set in the Code of Welfare for the particular species concerned.

Design criteria and choice of construction materials for livestock housing should meet the needs for the specific research and management practices. To the extent possible, all material used for indoor facilities should be impervious to moisture, insects and vermin. Concrete and metal are the preferred building materials. Wood can be satisfactory, but it must be properly painted and sealed if extensive cleaning and disinfection procedures are to be carried out.

Floors and other paved surfaces should have textures that minimise slipping and possible injury. Fencing should be properly maintained to prevent escape or injury. Ruminants require a resting area either in a well-drained outside area or bedded shelter. Control of air, temperature, relative humidity, air velocity, moisture, dust, light, gas accumulation, odours, space and manure become of increasing concern in shifting from ambient housing to a totally controlled environment.

When animals are fed in groups, there should be sufficient trough space or feeding points to avoid undue competition for food, especially if feed is restricted. Feeding space is determined by the size and number of animals that must eat at one time.

An adequate water supply is also essential. Water sources should be easily accessible to animals of all ages and designed to prevent faecal contamination. Equipment for provision of food and water should be constructed of materials that can be easily and effectively cleaned.
4.7 Enrichment and environmental complexity

Most animals used in research, testing and teaching are housed in unnatural environments. Wherever possible such animals should be provided with an environment that can accommodate the behavioural and physiological needs of the species.

Almost all the species of animals used in research, testing and teaching have well defined social structures and prefer to live in groups, although care must be taken to ensure that animals are socially compatible. Individual housing is stressful for social animals, and social isolation should be avoided whenever possible and limited to meet the specific research objectives as approved by an AEC. The effects of physical isolation should be minimised where possible by the use of non-contact communication, whether visual, auditory or olfactory. Judicious use of mirrors can also be helpful, as can an environment of increased complexity.

The living areas of the animals should be set up and provisioned with the means that will enable them to perform a behavioural repertoire appropriate to the species.
5.1 Management and personnel

5.1.1 Facility manager

The process of animal acquisition and the management of breeding and holding facilities should be supervised by persons with appropriate veterinary or animal care qualifications or experience with the species involved. The facility manager should ensure that adequate veterinary care is provided for all animals held for breeding and for experimental manipulations with ready access to institutional or consultant veterinary services seven days a week.

The facility manager should:

- be responsible for the management of the day-to-day care of the animals in holding and breeding facilities;
- supervise the work of other personnel in the facility;
- act as liaison between investigators and teachers and facility personnel;
- communicate with the AEC on management of the facility and any adverse incidents;
- contribute to the development and maintenance of the institution’s animal care policies and procedures;
- be knowledgeable regarding signs of pain, distress and illness specific to each species housed, and should ensure that the well-being of all animals is regularly assessed. (After animals are allocated to an approved project, the investigator has primary responsibility for ensuring adequate monitoring of the animals’ well-being);
- ensure that ill or injured animals which are not assigned to approved projects are treated promptly and that the cause of death is investigated for animals which die unexpectedly;
- ensure that personnel are provided with appropriate protective clothing, maintain high standards of personal hygiene and do not eat, drink or smoke in animal areas;
- document procedures used in the management of small animal holding and breeding facilities. These procedures should be approved as Standard Operating Procedures (SOPs) by the AEC and should take into account the requirements of the species held, the experiments being conducted, and the health and safety of the personnel, and include transport, quarantine and disposal of animals, routine husbandry, prevention, diagnosis and treatment of disease, monitoring of health status and genetic constitution, and physical environmental factors. These procedures should be made known to all personnel involved in the care and use of the animals and should be reviewed regularly. For large animals, transport and routine husbandry procedures as recommended in the relevant Codes of Welfare should be followed unless alternative procedures have been approved by the animal ethics committee;
- for small animal colonies, maintain a regular schedule of cage, equipment and facility sanitisation to ensure that potential pathogens are kept at minimum levels in the environment;
- ensure that adequate records are maintained of:
  - the source, care, allocation, movement between locations, use and disposal of all animals, and of any diseases developed;
  - the fertility, fecundity, morbidity and mortality in animal breeding groups, in order to monitor the management of the groups, and to assist detection of the origin and spread of disease; and
  - the health status, genetic constitution and the physical environment of the animals, when definition of these is required.

Records maintained by the facility manager must be made available to investigators.

The facility manager should ensure that investigators and teachers are informed of any changes to the conditions under which animals are held and which may affect their work.
5.2 Personnel

The most important factor ensuring high standards of animal care is a sufficient number of well-trained, committed staff. Personnel working with animals in a holding facility should be appropriately instructed in the care and maintenance of those animals, and in how their actions may affect the animals’ well-being and the outcome of experiments.

Institutions should encourage and promote formal training in animal science or technology (see Appendix 2 for a list of courses).

Personnel employed in the care of animals should be trained to recognise at an early stage changes in animal behaviour, performance and appearance.

New personnel who will care for animals should be appropriately instructed in their duties and in institutional policy and procedures.

Personnel should be informed of allergy hazards and the important zoonotic diseases of animals under their care and of precautions that should be taken. Regular health checks and appropriate immunisation (e.g. against tetanus and other zoonoses) of all personnel who handle animals are recommended in the interests of both personnel and animals.

5.2 Husbandry procedures

5.2.1 Food and water

Animals should receive appropriate, clean and nutritionally adequate food according to accepted requirements for the species. The food should be in sufficient quantity and of appropriate composition to maintain normal growth of immature animals or normal weight of adult animals and to provide for the requirements of pregnancy or lactation.

Uneaten perishable food should be removed promptly unless contrary to the needs of the species. Where possible, alteration to dietary regimes should be gradual.

When animals are fed in groups, there should be sufficient trough space or feeding points to avoid undue competition for food, especially if feed is restricted. Feeding space is determined by the size and number of animals that must eat at one time.

Drinking water should be constantly and reliably available, and be clean, fresh and uncontaminated.

5.2.2 Routine husbandry procedures

Routine husbandry procedures should comply with any code of welfare for the species involved and must be performed by competent personnel. Variations to normal procedure as part of an experimental project must receive prior AEC approval. Procedures applied to the maintenance of breeding stock and supply of animals are viewed as routine husbandry and fall outside the definition of manipulation. When special breeding requirements are integral to a research or teaching project such as in the creation of a genetically modified animal, then procedures applicable to breeding must be regarded as a manipulation and should be included in the proposal to the AEC.
5.3 Identification of animals

Animals, whether as individuals or groups, should be identified by a method such as tattoo, neck-band, individual tag, electronic numbering device, physical mark, or by a label or marking attached to the cage, container, pen, yard or paddock in which the animals are kept. The more invasive identification procedures should be performed, or closely supervised, by an experienced practitioner.

The method of identification should be reliable, appropriate for the species and project, and cause the least possible stress to the animal.

5.4 Disposal of animal carcasses and waste

Appropriate provision must be made for prompt and sanitary disposal of animal carcasses and waste material in accordance with HSNO and ERMA legislation, local council by-laws and community standards.
6.1 General

People who use animals for scientific purposes have an obligation to treat the animals humanely and to consider their welfare as an essential factor when planning and conducting experiments.

Investigators have direct and ultimate responsibility for all matters related to the welfare of the animals under their control, including the general husbandry and housing of those animals as well as the specific manipulations. They should act in accordance with their specific AEC approval. The responsibility of investigators extends over all facets of the care and use of animals in projects approved by the AEC, beginning when the animal is allocated to the approved project and ending with its fate at the end of the project. Investigators are responsible for the standard of animal care and use by all other persons involved in the project. They should ensure that the extent of supervision is compatible with the level of competence of each person and the responsibilities they are given.

However, it is recognised that in many institutions the duty of managing routine animal husbandry is delegated to professional animal care personnel on a daily basis. Strategies must be in place for the facility manager to effectively communicate with the investigator regarding animal welfare and research concerns.

Investigators have a legal and ethical responsibility to ensure that animals on a project are manipulated using medical and surgical techniques which are consistent with the principles of good practice and scientific knowledge in the discipline of laboratory animal veterinary medicine. Investigators should consult with veterinarians whenever unexpected adverse effects occur in order that standard veterinary care treatment regimes are immediately implemented. This responsibility parallels the public’s duty of care to seek veterinary management of any sick animals in their charge.

6.2 AEC approval

Before any project begins, investigators must submit a proposal to the AEC, which demonstrates that the project will comply with the Animal Welfare Act 1999. Moreover, the investigators must satisfy the AEC of their competence to conduct the techniques described in the experiment.

Investigators must not begin experiments before written AEC approval is obtained and must adhere to any requirements of the AEC.

Investigators may, however, obtain and hold for adaptation species which are not otherwise readily available, prior to formal AEC approval, provided that their research use does not commence until approval is given.

Animal ethics committees have responsibility for monitoring compliance with current legislation, codes of ethical conduct and the conditions set in AEC approvals in respect of the use of animals in research, testing and teaching.

Species requiring special permission from other organisations (e.g. Department of Conservation) must not be obtained and held before such approval is granted (see section 3, Acquisition of Animals).

Investigators should inform the AEC when each project is completed or discontinued. As part of its role in monitoring manipulations the AEC should also be informed of the outcome of the project.
6.3 Planning projects

It is essential that investigators carefully plan their experimental protocol. This guide raises important issues for consideration which may lead to adjustments to the final protocol for submission to the AEC.

**Choice of Animal** – Investigators 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, investigators must ensure that the supplier can provide adequate proof that any requirements can be met. Where relevant, species and individual animals should be chosen on the basis that the proposed experiments 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.

**Monitoring** – Investigators should ensure that all intensively managed animals are observed as frequently as circumstances require, but at least daily, to assess their health and welfare. Investigators should ensure that satisfactory arrangements are made for contacting them and other responsible persons in the event of emergencies. Any unexpected adverse effects that impact on animal welfare must be reported to the AEC.

**Record-keeping** – Investigators should ensure that their experimental research records include details of animal husbandry routine, environmental conditions, and other potential non-experimental variables which may affect the study. Monitoring records, including methods of assessment of health and welfare should also be maintained. Records must also meet the statistical reporting requirements of the Animal Welfare (Records and Statistics) Regulations 1999, as detailed in MAF’s publication *Animal Use Statistics*, which is supplied to all code holders.

**Consultation** – Investigators should consult other experienced scientists, veterinarians, or laboratory animal, livestock or wildlife specialists when necessary.

**Checklist** – When planning is completed, the investigator should re-check the protocol to ensure that the following points have been adequately covered and the Three Rs (replacement, refinement and reduction) have been considered:

a. Do the potential benefits outweigh any ethical concerns about the impact on animal welfare?

b. Can the aims be achieved without using animals (replacement)?

c. Are there better ways of achieving the same ends (refinement)?

d. Are suitable holding facilities, equipment and competent personnel available (refinement)?

e. Have all involved personnel been informed of the planned experimental and other procedures?

f. Has the most appropriate species of animal been selected?

g. Is the biological status (genetic, nutritional, microbiological, general health) of the animals appropriate?

h. Are the environmental conditions (including caging or pen type, noise, photoperiod, temperature, humidity, ventilation, density of housing and social structures) appropriate (refinement)?

i. Are the experiments designed so that statistically valid results can be obtained or the educational objectives achieved using the minimum necessary number of animals (reduction)?

j. If the potential impact on the animal is unknown, is it appropriate to incorporate a pilot study into the project design to allow a staged assessment of the impact on animal welfare and how it will be managed (refinement)?
k. If the scientific activity could cause the animals any pain or distress, what will be done to minimise or avoid this (refinement)?

l. What arrangements will be made to monitor the animals adequately, in terms of both their general health and welfare and their response to manipulation (refinement)?

m. If any of the experiments have been performed previously, why should they be repeated (reduction)? Is this required for quality control or legislative reasons (repetition, replication)? Have you checked to see if similar work has been done before so that you can avoid duplicating previous work and thus using animals unnecessarily?

n. If any animals are to be used repeatedly, what will be done to minimise the cumulative effects of such use (refinement)?

o. Are there any permits that must be obtained for the importation, capture, use, destruction or release of the animals?

p. What arrangements have been made for the fate of all healthy animals at the completion of the project?

### 6.4 Conduct of experiments

#### 6.4.1 Limiting pain and distress

Pain and distress cannot always be adequately evaluated in animals and investigators must therefore assume that animals experience pain in a manner similar to humans. Decisions regarding their welfare in experiments must be based on this assumption unless there is evidence to the contrary.

The investigator should anticipate any potentially adverse effects of a manipulation and take all possible steps to avoid or minimise pain and distress.

These steps should include:

- choosing the most appropriate and humane method for manipulations;
- ensuring the technical skills and competence of all persons involved in animal care and use;
- using pre-emptive analgesia when pain is anticipated;
- ensuring that animals are adequately monitored to allow prompt alleviation of pain or distress;
- developing a plan to manage any adverse effects of a manipulation e.g. increase in frequency of observation, consultation with a veterinarian, administration of appropriate medication, removal from the project or humane euthanasia;
- using anaesthetic, analgesic and tranquillising agents appropriate to the species and the experimental purposes;
- developing study endpoints that minimise pain and distress;
- conducting projects over the shortest time practicable; and
- using appropriate methods of euthanasia.

The use of local or general anaesthetics, analgesics or tranquillisers must be appropriate to the species, and should meet the criteria generally accepted in current medical, laboratory animal or veterinary practice.

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

Distress can sometimes be avoided or minimised by non-pharmacological means. Before an experiment begins, animals should be appropriately conditioned to the experimental environment and procedures and be familiar with animal care personnel. During and after experiments appropriate nursing procedures to
minimise pain and distress and to promote the well-being of the animals should be provided.

If animals develop signs of severe pain or distress despite the precautions outlined above, they should have the pain or distress alleviated promptly or must be killed humanely and without delay. Veterinary consultants involved in the animal care programme should be informed immediately. Alleviation of such pain or distress takes precedence over continuing or finishing the experiment. If in doubt, investigators must always seek a professional veterinary opinion before continuing an experiment.

Unexpected deaths occurring during a project must be properly investigated by a veterinarian or other qualified person to determine the cause and initiate remedial action. If the deaths are due to manipulations, these must cease. The AEC must be notified and the project protocol resubmitted with appropriate modification.

6.4.2 Animal welfare monitoring of pain or distress

Investigators should be familiar with the normal behaviour of the animal species chosen, be knowledgeable of signs of pain or distress specific to that species, and must regularly monitor their animals for these signs.

Animals should 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. Any changes in patterns of sleeping, feeding, drinking, grooming, exploratory behaviour, performance in learning or discriminatory tasks, reproduction or social behaviour should be noted, assessed and acted on if appropriate.

Animals must be monitored appropriately for clinical signs of acute pain or distress. (See Appendix 1 for further information on pain).

Animal welfare monitoring score sheets should be used to document the observations and collection of data listed above. For example, variations in body weight, water intake, and grooming behaviour can be recorded every 24 hours or more often as needed during the immediate post-operative period for surgical manipulations. A sample monitoring sheet is included as Appendix 3.

6.4.3 Study endpoints

For all but the most minor of manipulations, the investigator should develop humane study endpoints when preparing a project application. These can be used to judge when an animal should be removed from a project or euthanased in order to promote animal welfare.

Death as an endpoint is generally unacceptable and should be fully justified. All animals found in a moribund state must be euthanased unless specifically justified as above and approved by the AEC. It is generally accepted that moribund animals give unreliable research data because they are frequently in a state of multiple organ failure. However, attention to good practice indicates that endpoints earlier than the moribund condition should always be used. Typically these are based on changes in body weight, tumour size, and/or body temperature combined with abnormal clinical condition.

For example, animals should be killed when:

- they have lost more than 20 percent of their pre-study body weight; or
- they have lost more than 10 percent in 24 hours; or
- a tumour grows to more than 10 percent of the animal’s weight; or
- body temperature falls below a pre-set level (as determined by pilot studies which indicate that the level set is predictive of death); or
- animals self-mutilate limbs and feet; or
- animals develop abscesses.
6.4.4 Repeated use of animals in experiments

Individual animals should not be used in more than one experiment, either in the same or different projects, without the express approval of the AEC. However, appropriate re-use of animals may reduce the total number of animals used in a project, result in better design of experiments, and reduce stress or prevent pain in other animals.

When approving projects involving the re-use of animals, the AEC should take into account:
- the pain or distress and any potential long-term or cumulative effects caused by any previous manipulations;
- the total time taken;
- the pain or distress likely to be caused by the next and subsequent manipulations; and
- whether the animal has recovered fully from the first experiment before further experiments are carried out.

6.4.5 Duration of experiments

Experimental duration should be the minimum required to provide answers to the questions asked.

Experiments, particularly those which involve any pain or distress, should be as brief as practicable. AEC 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.

6.4.6 Handling and restraining animals

Animals should be handled only by persons instructed and competent in methods which minimise pain, injury or distress.

When the use of restraint devices is necessary for the welfare of the animal and the safety of the handler, they should be used to the minimum extent, for the minimum period required to accomplish the purpose of the experiment, and be appropriate for the animal.

Tranquilisers or anaesthetics may aid restraint but may prolong recovery from the procedure. When these agents have been used, greater attention may be required in assessing the recovery of the animals.

Periods of prolonged restraint or confinement should be avoided. However, where prolonged restraint or confinement is proposed, such as housing livestock in metabolism cages, consideration should be given to their biological needs, including their behavioural requirements and the need for appropriate exercise. Such animals must be assessed regularly by a veterinarian or other qualified person not otherwise involved in the project. If any negative impact on an animal is detected, the animal should be removed from the restraint or the method modified to minimise the impact.

6.4.7 Completion of projects

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, when necessary, be euthanased.

Where practicable, investigators should share with other investigators tissue from animals being euthanased.

6.4.8 Euthanasia

When it is necessary to kill an animal, humane procedures must be used. These procedures should fulfill the following requirements:
• They should be minimally stressful.
• They should be reliable.
• They should produce rapid loss of consciousness without pain until death occurs.
• The appropriate means must be readily at hand.
• The procedures should be compatible with the aims of the experiments.
• The procedures should be performed only by persons who have demonstrated to a veterinarian or other qualified person that they are competent in the methods to be used.
• 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.
• Dependent neonates of animals being killed must also be killed or provision made for their care.
• Methods of killing must be appropriate to the developmental stage of the animal. Disposal of fertilised eggs, fetuses and embryos must not occur until death is confirmed.

Investigators are referred to the monograph *Euthanasia of Animals Used for Scientific Purposes* published by the Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCRRCA) or the AVMA Guidelines for Euthanasia at http://www.avma.org/issues/animal_welfare/euthanasia.pdf for specific recommendations for euthanasia of the various species of laboratory animals.

6.4.9 Autopsy

An autopsy should be performed by a person with appropriate qualifications when animals die unexpectedly or are euthanased due to unforeseen complications. Investigators should consider the value of an autopsy for such animals. Post-mortem evaluation may identify one or more non-experimental variables which could compromise the remaining research subjects.

6.4.10 Pre-operative planning

Surgical success can be improved by careful attention to the following:
• The use of animals that are fit for purpose will ensure more reliable research data. Investigators should consult the institutional veterinarian or other qualified person to assist in obtaining such animals.
• Pre-operative physical examination can often identify potential problems, such as increased anaesthetic risk, which may compromise the surgical procedure. Animals that are not in an appropriate state of health should be rejected.
• Pre-surgical fasting is necessary for many species to minimise complications of anaesthetic administration.
• Pre-operative antibiotic administration should be considered. This can ensure maximal blood levels of drug during the surgical procedure. Additional post-operative antibiotic treatment may be required.
• Pre-experimental practice on cadavers enables investigators to familiarise themselves with anatomical landmarks and streamline the experimental surgical procedures, thereby reducing the quantity of anaesthetic required, reducing operative time and minimising tissue damage. This will speed post-operative recovery and promote animal well-being.
• For any surgical procedure, a pain management plan aimed at prevention or alleviation of pain and which is appropriate for the procedures and the species should be developed, implemented and reviewed if necessary.

6.4.11 Surgery

Anaesthesia and surgery should be performed by competent personnel with appropriate training and experience. Training should be given by competent personnel with relevant expertise in surgery and anaesthesia.
Surgical procedures should be carried out under appropriate local or general anaesthesia. There should be adequate monitoring of the depth of anaesthesia and effects such as hypothermia, and cardiovascular and respiratory depression.

The choice and administration of anaesthetic, analgesic and tranquillising agents should be suitable for the species and appropriate for the purpose of the experiment. The use of such agents should parallel that used in current medical, laboratory animal or veterinary practice.

Investigators should consider the value of a limited anaesthetic trial to familiarise themselves with new anaesthetic or analgesic drug combinations. Species and strain variation in drug metabolism can result in unexpected morbidity and mortality when dosages are extrapolated from published data. A limited trial, when combined with a non-survival surgical practice session, can provide invaluable information and promote surgical success and animal well-being in subsequent study animals.

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

When the animal is not to recover from the surgery, it must be unconscious for the whole procedure, euthanising either by overdose of the general anaesthetic or by inducing brain death by a variety of methods e.g. exsanguination or disruption of the thorax.

When the animal is to recover from the anaesthetic, surgical procedures must conform to accepted standards in laboratory animal and veterinary practice. All tissues should be handled with care and particular attention should be given to haemostasis. Aseptic technique should be used for animals which undergo any survival surgery which involves disruption of the skin’s barrier function. Aseptic technique includes aseptic preparation of the surgical field, use of sterilised instruments, wearing of sterile surgical gloves, gowns, caps, and face masks. The use of post-operative antibiotics should not be a substitute for correct aseptic technique.

6.4.12 Post-operative care

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. Consideration of pain relief is paramount in post-operative care. The use of analgesics and tranquillisers may be needed to minimise post-operative pain or distress. Care should be taken that animals recovering from anaesthesia are housed to prevent injury and that conditions are such that they are not disturbed, attacked or killed by other animals in the same enclosure.

Investigators should ensure that animals are adequately monitored. They should ensure that they, or other experienced personnel, are fully informed of the animals’ condition. The duties of all personnel must be clearly defined and ways of dealing with emergencies established.

Appropriate clinical records, including observations and administration of any drugs, fluids or other treatments, should be kept, and made accessible to all involved in the post-operative care of the animal.

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

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 and the consultant veterinarian informed immediately.
6.4.13 Implanted devices

Investigators should be aware of the need for strict attention to aseptic technique when foreign bodies are surgically implanted. Contamination of prosthetic devices frequently requires their removal after antibiotic therapy has failed.

Skilled and specialised 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.

6.4.14 Organ and tissue transplantation

Skilled and specialised attention is required for animals following organ or tissue transplantation. Animals must be assessed frequently for any signs of pain, distress, infection and tissue rejection and treated immediately if these occur. Special attention should be given to the management of immunosuppression and the disease hazards and adverse outcomes that may be associated with organ and tissue transplantation between species (xenotransplantation). Death as an endpoint is unacceptable when determining recipient survival times.

6.4.15 Neuromuscular paralysis

Neuromuscular blocking agents may only be used with adequate general anaesthesia. When these agents are used, specialist advice on anaesthesia should be obtained.

Immobilisation of an animal solely with a neuromuscular blocking agent is not acceptable. When these agents are used with general anaesthesia, special care must be taken to ensure the maintenance of an adequate plane of anaesthesia. Since the usual criteria such as 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, oxygen saturation, pupil size and electroencephalogram is necessary, together with the effects of these of mild sensory stimuli. Care is required to ensure that drugs used in the procedures do not interfere with this monitoring.

6.4.16 Electroimmobilisation

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

6.4.17 Animal models of disease

The scientific validity of animal models of human diseases rests in part on how closely a given model resembles a particular disease. Thus, the attendant pain and distress of the human disease 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 minimised and that the AEC is informed of the potential effects of the disease on the animals.

Investigators must not allow the experiments to proceed to the painful or distressful or lingering death of animals unless no other experimental endpoint is feasible and the goals of the experiments are the prevention, alleviation, treatment or care of a life-threatening disease or situation in human beings or animals.

Investigators must avoid using death as an experimental endpoint whenever possible. When death as an endpoint cannot be avoided, the experiments must be designed to result in the deaths of as few animals as
6.4.8 Modifying animal behaviour

Procedures used to modify an animal’s behaviour or to induce it to perform specific tasks depend on motivating the animal. The preferred inducement is positive reinforcement, but in some cases the inducement may need to be some form of biological stress. This stress should be as mild as possible. Severe deprivation of water, food, social interaction or sensory stimuli should not be used.

The level and duration of painful or noxious stimuli should be minimised and escape from the stimuli should be available. Behaviour can usually be modified using procedures that involve no more of a stressor than that normally experienced by the species. When noxious stimuli are used to modify behaviour the AEC should be aware of the duration and possibility of escape from the stimuli.

6.4.9 Toxicological experiments

Investigation of the safety of agents intended for use in human beings, animals, the household or the environment, or of naturally occurring toxins, or of agents to be used as poisons for pest control 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.

The endpoint of such experiments must be as early as is compatible with reliable assessment of toxicity, and must minimise the extent of any pain and distress.

Investigators must not allow the painful, distressing or lingering deaths of animals unless no other endpoint is feasible and the goals of the project are the prevention, alleviation or cure of a life-threatening disease or situation in humans or animals.

When death as an endpoint cannot be avoided, the experiments must be designed to result in the deaths of as few animals as possible. (Refer to section 6.4.3.)

6.4.20 Experiments involving hazards to humans or other animals

Hazards may arise from sources that include:

- viruses;
- bacteria;
- fungi;
- parasites;
- radiation;
- radioactivity;
- corrosive substances;
- toxins;
- allergens;
- carcinogens;
- recombinant DNA;
- anaesthetic gases; and
- physical injuries.

Any potential pathogenic effects of these hazards when used in experiments must be explained to the fullest extent possible to all personnel. Tests before, during and after the experiments may be required for personnel.

The investigator should inform the AEC that the advice of the institution's biological safety committee or equivalent (where it exists) has been sought and that appropriate measures for containment, disposal and
decontamination have been established.

Protocols submitted to the institution's AEC should include a description of any intended use of hazardous compounds or organisms. They should describe specific safety measures and disposal protocols used to prevent contamination of caging, other animals, research personnel and students.

Investigators must not allow the experiments to proceed to the painful or distressful or lingering death of animals unless no other experimental endpoint is feasible and the goals of the experiments are the prevention, alleviation, treatment or care of a life-threatening disease or situation in human beings or animals. When death as an endpoint cannot be avoided, the experiments must be designed to result in the deaths of as few animals as possible. (Refer to section 6.4.3.)

Precautions, security and emergency plans to contain hazardous agents must be appropriate to a “worst-case” situation.

6.4.21 Experimental manipulation of animals’ genetic material

All projects involving the introduction of foreign DNA into mammalian cells or whole animals must be conducted in accordance with requirements and guidelines issued by the Environmental Risk Management Authority.

The definition of an animal under the Animal Welfare Act 1999 excludes mammalian fetuses in the first half of their gestation period or developmental stage; avian or reptilian pre-hatched young in the first half of their gestation period or developmental stage; and pre-pouch marsupial young. In addition, all other animals in the pre-natal, pre-hatched, larval, or such other developmental stages are not considered to be animals for the purposes of the Act (for example, fish and amphibians are not animals until they are hatched). It follows that these are not covered by the Act, although the following points should be noted:

- In mammals the acquisition of eggs following superovulation and the re-implanting of those eggs following modification, is itself a “manipulation” of the mother. Therefore the procedure requires approval from an AEC.
- In egg-laying avian or reptilian animals the collection of eggs is a normal procedure and any treatment of those eggs in the first half of gestation would not be a manipulation according to the Act. But if such treated eggs are held and then manipulated in the second half of gestation (or after they hatch) AEC approval will be required for manipulation at that stage of development.
- In egg-laying animals (other than avian or reptilian animals) any treatment of the eggs would not be a manipulation according to the Act. If such treated eggs are held and hatched, then any subsequent manipulation requires AEC approval.
- Where a fetus or pre-hatched young becomes an animal (either by reaching the second half of its gestation period or by “emerging”) and there is no subsequent manipulation of the animal, the animal will still be covered by the general provisions of care in Parts 1 and 2 of the Act.
- Any manipulation subsequent to legal status of “animal” being attained must be submitted to the AEC as usual.

The potential impact of the introduction of a new gene, or the alteration of the expression of existing genes on all animals should be considered. The investigator should inform the AEC of any potential side-effects of genetic manipulation that may impact negatively on the welfare of the parent animal or its offspring, and of the means that will be used to deal with such eventualities. Details of monitoring for unexpected adverse effects arising from the genetic modification must be provided.

Proposals for the creation of genetically modified animals that are expected to suffer pain or distress must define special needs and give details of specialist care that will be provided to minimise these negative impacts. Humane endpoints must also be defined.
The clinical status of genetically modified animals may deviate unexpectedly from the predictions made in the proposal made to the AEC. Investigators must assess, through detailed monitoring, the welfare and genetic stability of newly created genetically modified animals and their offspring across a number of generations. The frequency of reporting such observations to the AEC should be determined by the AEC, taking into account the nature of the genetic modification.

6.4.22 Cloning of animals

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

6.4.23 Experimental induction of neoplasia

The site for induction of tumours must be chosen carefully. Subcutaneous sites on the back or flank should be chosen when possible. Prior to the use of footpad, brain and eye sites, specific justification as to the lack of any alternative should be made to the AEC.

Investigators should monitor their animals regularly for signs of pain or distress, especially sudden changes in body condition and signs that tumour growth is impacting on the wellbeing of the animals.

Animals with experimentally induced tumours should be euthanased before predictable death occurs, cachexia becomes advanced or the tumour becomes large enough to cause ulceration or severe limiting of normal behaviour. Animals should be used when tumours are at the minimum size necessary to obtain valid results.

In tumour therapy experiments, the endpoints chosen should be as early as possible, compatible with reliable assessment of the therapy. Weight changes should be monitored closely and criteria under 6.4.3 apply. Death from the tumour should not be an endpoint.

6.4.24 Production of monoclonal antibodies

In vitro methods should be used for the routine amplification of hybridomas for the production of monoclonal antibodies. Investigators wishing to use the in vivo (ascites) method should provide in their proposal to the AEC recent laboratory evidence to show that in vitro methods are unsuitable for the specific monoclonal antibody that is the subject of the proposal.

In the immunisation phase, investigators must ensure the minimisation of pain and distress to animals from factors such as the type, volume, site and frequency of the injection of adjuvants, and the methods and frequency of blood sampling.

With ascitic tumours, including hybridomas, investigators should 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. Careful monitoring is necessary because body weight loss can be difficult to discern in the presence of ascites and abdominal tumours.

6.4.25 Lesions of the central nervous system

Anatomical or chemical lesions of the central nervous system have been widely used to study its structure and function in health and disease. These experiments demand special consideration when the lesion produces loss of function, including 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 injury mechanisms. Special animal care, caging, and other facilities may be needed and the AEC, in approving such experiments, has a particular responsibility to ensure that these facilities are available and that the condition of the animals is closely monitored.

6.4.26 Withholding food or water

Projects involving the withholding or severe restriction of food or water should produce no continuing detrimental effect on the animals. In these experiments, the fluid balance and/or body weight must be monitored, recorded and maintained within the limits approved by the AEC.

6.4.27 Fetal experimentation

Under the Animal Welfare Act 1999, the definition of an animal includes mammalian, avian and reptilian prenatal or pre-hatched young in the second half of their developmental stage. Any projects involving such animals require AEC approval.

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

Although there is increasing evidence that fetuses do not feel pain under normal conditions, investigators should ensure adequate anaesthesia for both mother and fetus when the latter is undergoing surgical or other manipulation in utero.

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

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

6.4.28 Research on pain mechanisms and the relief of pain

In experiments in which unanaesthetised animals are to be subjected to stimuli designed to produce pain or when pain is to be inflicted on animals as part of normal management, investigators should:

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

6.4.29 Animal welfare and animal health research

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

- the principal aim of the project is to improve animal health or welfare;
- alternative methods, such as the use of animals already experiencing the problem, are not possible;
- all possible steps are taken to minimise any pain or distress; and
- the experiments do not proceed to the painful or distressful or lingering death of animals unless no other experimental endpoint is feasible and the goals of the experiments are the prevention, alleviation, treatment or care of a life-threatening disease or situation. When death as an endpoint cannot be avoided, the experiments must be designed to result in the deaths of as few animals as possible. (Refer to section 6.4.3.)
Animals should only be used for teaching activities when there are no suitable alternatives for achieving all of the educational objectives. Students should be given the opportunity to discuss the ethical, legal, social and scientific issues involved in the use of animals for scientific purposes, including teaching.

### 7.1 Tertiary institutions

When animals are being used to achieve educational objectives the person in charge of the class must:

a. accept ultimate responsibility for ensuring that the care and use of the animals is in accordance with this guide and all relevant legislation;

b. have relevant training and qualifications;

c. incorporate into the proposed activities any methods for the replacement, reduction or refinement in the use of animals, provided such methods are compatible with the educational objectives;

d. obtain prior AEC approval for use of all animals for the entire course and ensure that activities are conducted as directed and approved by the AEC;

e. instruct students appropriately in the care and use of animals before those students participate in experiments with live animals and, where possible, use alternative methods in that preparation;

f. ensure that there is close, competent supervision of all students;

g. allow students to anaesthetise animals or carry out surgery only if it is essential for their training;

h. ensure that in the event of injury to animals, treatments ranging from a minor procedure to euthanasia are available; and

i. be responsible for the humane killing of the animals, if required.

### 7.2 Secondary and primary schools

Use of animals in secondary and primary schools must comply with the approved code of ethical conduct of the New Zealand Association of Science Educators. This code covers early childhood centres, kindergartens, schools (both teachers and students) home-schooled students and their families and is administered by the association on behalf of the Ministry of Education. An animal ethics committee is established under the code. It approves appropriate projects and is able to provide advice on when AEC approval is necessary.

Any animals that are housed at schools must be well cared for at all times, including on weekends and holidays, in accordance with this guide or the relevant code of welfare for the species. Students should not be allowed to take animals home unless there is a clear, written undertaking from a parent or guardian that the animals will be cared for adequately and responsibly.

There are two resources of particular relevance to schools. One is *Ethical guidelines for students in laboratory classes involving the use of animals and animal tissues*, an ANZCCART publication. The other is *Caring for Animals – A Guide for Teachers, Early Childhood Educators and Students*, published by the Ministry of Education.
Published material on the use of animals for scientific purposes and alternatives to animal use is being extended almost daily. A bibliography would be too extensive to publish in this code. However, a minimal list would include the following:

- MAF (2009) *Guidelines for the welfare of livestock from which blood is harvested for commercial and research purposes*
- National Health and Medical Research Council (2004) *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (7th ed.).

Institutional libraries will have access to bibliographies through database searches.

### Useful websites

- Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART)  
- National Animal Ethics Advisory Committee (NAEAC)  
- Using Animals in Science  
  [anzccart.rsnz.org/](http://anzccart.rsnz.org/)
- American Association for Laboratory Animal Science (AALAS)  
  [www.aalas.org](http://www.aalas.org)
- Animal Welfare Information Centre (AWIC)  
- Animal Use in Veterinary Medical Education  
  [www.cvmbs.colostate.edu/cvmbs/animalabuse.html](http://www.cvmbs.colostate.edu/cvmbs/animalabuse.html)
- Biomedical Research Education Trust  
  [www.bret.org.uk](http://www.bret.org.uk)
- Canadian Council for Animal Care  
- FRAME  
  [www.frame.org.uk](http://www.frame.org.uk)
- Institute for Laboratory Animal Resources (ILAR)  
  [dels.nas.edu/ilar/](http://dels.nas.edu/ilar/)
- Iowa State University Bioethics Institute  
  [www.bioethics.iastate.edu/outreach.html](http://www.bioethics.iastate.edu/outreach.html)
- NC3Rs, National Centre for Replacement, Refinement and Reduction of Animals in Research, UK  
  [www.nc3rs.org.uk](http://www.nc3rs.org.uk)
- Nuffield Council on Bioethics  
  [www.nuffieldfoundation.org/bioethics/index.html](http://www.nuffieldfoundation.org/bioethics/index.html)
- Understanding Animal Research  
  [www.understandinganimalresearch.org.uk](http://www.understandinganimalresearch.org.uk)
- Universities Federation for Animal Welfare (UFAW)  
  [www.ufaw.org.uk](http://www.ufaw.org.uk)
- University of British Columbia Animal Welfare and Ethics Centre  
  [www.agsci.ubc.ca/animalwelfare](http://www.agsci.ubc.ca/animalwelfare)
- University of Nottingham Centre for Applied Bioethics  
  [www.nottingham.ac.uk/bioethics/#bioeth](http://www.nottingham.ac.uk/bioethics/#bioeth)
- AVMA Guidelines on Euthanasia (June 2007)  
  [www.avma.org/resources/euthanasia.pdf](http://www.avma.org/resources/euthanasia.pdf)
Alternatives

- www.go3r.org/ – search engines for finding alternatives to animal use in research, testing and teaching
- NORINA Database (contains information on audiovisuals and other alternatives/supplements to the use of animals in teaching) oslovet.veths.no/NORINA/
“What level of pain do we allow?” is a question facing all animal ethics committees. It is a matter which involves constant assessment of pain and stress levels of proposed research. The AEC must somehow reconcile the physical and psychological consequences for the animal with the objectives of the proposed investigation.

The concept of pain is subjective and difficult to define. The leading research journal *Pain* applies the following criterion:

*Pain in animals is manifested by abnormal behaviour which can be alleviated by analgesic procedures which relieve pain in humans.*

Flecknell, states: *Until further progress is made in assessing the nature of pain in animals, it should be assumed that if a procedure is likely to cause pain in man, it will produce a similar degree of pain in animals.*

The Swiss Academy of Sciences has outlines a set of Ethical Principles and Guidelines for Scientific Experiments on Animals. One of their statements is:

*Experiments apt to cause the animal severe suffering must be avoided by modifying the hypothesis to be tested in such a way that the other criteria of the successful conclusion of the experiment can be applied, or by foregoing the anticipated gain of knowledge. Severe suffering is to be construed as any state which in man would be qualified as unbearable without palliative measure.*

**2. Pain and distress**

**Pain** is an awareness of acute or chronic discomfort, occurring in varying degrees of severity, and resulting from injury, disease, or emotional distress as evidenced by biological or behavioural changes or both.

**Acute pain** results from a traumatic, surgical or infectious event that is abrupt in onset and relatively short in duration. It is generally alleviated by analgesics.

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

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

**3. Analgesia and anaesthesia**

A working knowledge of the following terms will be beneficial to AEC members to better understand pain in animals.

**Analgesia** refers to prevention of pain or relief of pain.

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1 Flecknell, PA “The Relief of Pain in Laboratory Animals”, *Lab Animals* 18: 147–160, 1984
Tranquillisation is a state of behavioural change in which the patient is relaxed and unconcerned by its surroundings. In this state the animal is often indifferent to minor pain.

Sedation is a mild degree of central depression in which the patient is awake but calm. Narcosis in humans is defined as a drug-produced state of deep sleep accompanied by analgesia. In veterinary medicine, the narcotised patient is seldom asleep but is sedated and oblivious to moderate pain.

Hypnosis is a condition of artificially induced sleep, or a trance resembling sleep, resulting from moderate depression of the central nervous system.

Local anaesthesia is the loss of sensation in a limited area of the body.

Regional anaesthesia is insensibility in a larger but limited area of the body.

Basal anaesthesia is a light level of general anaesthesia usually produced by pre-anaesthetic agents. It serves as a basis for deeper anaesthesia on administration of other agents.

General anaesthesia is complete unconsciousness.

Surgical anaesthesia is unconsciousness accompanied by muscular relaxation to such a degree that surgery can be performed painlessly without struggling on the part of the patient.

4. Signs of pain

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

- attraction to the area of pain;
- increased skeletal muscle tone;
- altered electroencephalogram (EEG) response;
- increased blood pressure and heart rate;
- pupillary dilation;
- change in the respiratory system.

(a) Signs of acute pain

- protection of the painful part;
- vocalisation (especially on movement or palpation of painful area);
- licking;
- biting;
- scratching or shaking of affected area;
- restlessness;
- sweating;
- increased rate of respiration;
- unusual immobility or reduction in mobility.

(b) Signs of chronic pain

- limping (if painful part is an appendage);
- licking of area affected;
- licking of other areas if the painful part cannot be reached;
- reluctance to move;
- loss of appetite;
- change in personality;
- change in eye brightness.
(c) Species specific signs

In compiling general guidelines it has become clear that there are species specific signs of pain which should be taken into account when making a practical assessment. Experience has taught that such signs are often associated with what is believed to be a painful condition, although no sign can by itself be regarded as diagnostic of pain and may also occur in conditions in which pain is unlikely to be a feature.

Although a comprehensive description of species specific signs has not been produced, the following notes and comments might be helpful.

(i) Primates

(Primates are not currently used in research in New Zealand. However, for completeness the following is included).

Monkeys often show remarkably little reaction to surgical procedure or to traumatic injury. Obvious signs of pain are not readily seen. Loud and persistent vocalisation is unlikely to be an expression of pain, but it is more likely to signify alarm or anger.

Pain gives rise to a general appearance of misery and dejection. The animal may be huddled in a crouching posture with a “sad” facial expression and glassy eyes; it may moan, avoid its companions and may stop grooming itself. A monkey in pain may also attract increased attention from its cage mates. This can vary from social grooming to attack.

Acute abdominal pain may be shown by facial contortions, clenching of teeth, restlessness and shaking accompanied by grunts and moans. Food and water are usually refused.

(ii) Horses

The following signs are associated with pain: periods of restlessness; interrupted feeding with food held in the mouth uneaten; anxious appearance with dilated pupils and glassy eyes; increased respiration and pulse rate with flared nostrils; profuse sweating and a rigid stance.

In prolonged pain, behaviour may change from restlessness to depression with head lowered. In pain associated with skeletal damage, limbs may be held in unusual positions and there is a reluctance to move with head and neck “fixed”. In abdominal pain, a horse may look at, bite or kick its abdomen; it may get up and lie down frequently; walk in circles; roll and injure itself as a result of these activities. This state may progress and can last for several hours. When near collapse, the horse may stand very quietly rigid and unmoving. Horses in pain generally show a reluctance to be handled.

(iii) Cattle

Cattle in pain often appear dull and depressed with little interest in their surroundings. There is loss of appetite, weight loss and, in milking cows, a sudden drop in milk yield. Severe pain often results in rapid shallow respiration. On handling they may react violently or adopt a rigid posture designed to immobilise the painful region. Grunting and grinding of teeth may be heard. Generally signs of abdominal pain are similar to those seen in the horse but are less marked. Rigid posture may lead to a lack of grooming because of an unwillingness to turn the neck.

(iv) Sheep and Goats

In general, signs of pain in sheep and goats are similar to those in cattle. Changes in posture and movement are apparent and a change in facial expression may be indicative of pain. Goats in particular are more likely than cattle to vocalise in response to pain. Grinding of teeth and grunting are also heard.

(v) Pigs

Pigs in pain may show changes in gait and posture. Pigs normally squeal and attempt to escape when
handled but these reactions may be accentuated when in pain. Pigs will often be unwilling to move and may hide in bedding if possible.

(vi) Dogs
Dogs in pain generally appear quieter and less alert with stiff body movements and an unwillingness to move. In severe pain the dog may lie still and adopt a crouching attitude. In less severe states it may appear restless. There may be loss of appetite and shivering and increased respiration with panting. Spontaneous barking is unlikely, the dog is more likely to whimper or howl, especially if unattended and may growl without apparent provocation. A dog may bite or scratch at painful regions and may become more vicious when handled.

(vii) Cats
Cats in pain are generally silent, but may growl or hiss if approached. There is loss of appetite and a tendency to hide. Posture becomes stiff and the cat may sit hunched lying on its chest being reluctant to stretch out. A cat in severe pain may howl and show demented behaviour and desperate attempts to escape. Incessant licking is sometimes associated with pain. More usually the cat has a generally miserable ungroomed appearance with a change from its normal temperament. There may be panting with an increased pulse rate and dilation of the pupils.

(viii) Rabbits
Rabbits in pain may be apprehensive, dull, inactive and assume a ‘hunched’ appearance. They sometimes, however, show aggressive behaviour and activity may be increased with excessive scratching and licking. Reactions to handling are exaggerated and acute pain may result in vocalisation. Respiratory rate may be increased and there may be loss of appetite.

(ix) Laboratory Rodents
The six classical symptoms of pain observed after laparotomy studies in rats are:
- writhing;
- arching;
- staggering;
- belly-press;
- falling; and
- twitching.
In addition, pain in rodents usually results in decreased activity, hair standing on end and an ungroomed appearance or there may be excessive licking and scratching. They may become unusually aggressive when handled, and acute pain may cause vocalisation. Loss of appetite or a change in feeding activity may be noted and, if housed with others, a change in the normal group behaviour may be apparent.

(x) Birds
Birds in pain may show escape reactions with vocalisation and excessive movement. There may be an increase in heart and respiratory rates. Prolonged pain will result in loss of appetite and inactivity with a drooping miserable appearance. When handled the escape reaction may be replaced by a state of rigidity.

(xi) Fish
It is difficult to determine the nature of the response to pain in fish. Responses to harmful stress include an increased breathing pattern with excessive movement of fins.
5. Painful procedures

A report on pain published by the Royal Society for the Prevention of Cruelty to Animals (England)² lists the following procedures to experimental animals as cause for concern:
- application of painful stimuli;
- creation of short or long-term inflammation (paws, joints);
- creation of animal models of human disease (renal failure, gastric and colonic ulcers, viral and bacterial infections, disorders affecting deep sea divers);
- transplantation studies;
- creation of tissue damage by radiation, often involving death as endpoints;
- induction of convulsions by electroshock to the brain;
- application of inescapable electroshock to feet or eye region;
- exposure to stress (cold, restraint, forced swimming);
- food deprivation for long periods,
- maternal deprivation of infant primates;
- toxicological studies involving severe and prolonged symptoms of illness, often ending in death;
- administration of substances (possibly hallucinogenic in humans) causing disorientation or abnormal behaviour;
- trauma research (burning, scalding, limb fracture, limb ischaemia);
- testing antidote to biological warfare agents;
- vaccine quality control assays involving death endpoints.

6. NAEAC’s views on pain relief

It is the view of NAEAC that animals should always be given the benefit of any doubt concerning pain relief.

Experiments which cannot be performed without unrelieved pain and distress should not be undertaken. Some experiments, even when some “good” results per se may be hoped for, should be forsaken altogether rather than subject animals to prolonged suffering or to intense pain even for short periods of time.

NAEAC concurs with the Canadian Council on Animal Care³ which classes as unacceptable:
- the utilisation of muscle relaxants or paralytics alone, without anaesthetics, during surgical procedures; and
- traumatising procedures involving crushing, striking or beating in unanaesthetised animals.

NAEAC is concerned about the present use and knowledge of effective analgesia for laboratory animals. There appears to be inadequate or contradictory data on the therapeutic effects of certain analgesic drugs for laboratory animals, and in particular, for small rodents which comprise the largest number of animals. Small rodents are rarely given analgesia.

According to Dr Eugene M Wright, et al,

…analgesic drugs are often withheld because the clinician is not absolutely certain that an animal is experiencing pain, yet antibiotics are almost always given, even without documenting the presence of bacterial infection. Pain and suffering may actually constitute the only situation in which one should go ahead and treat even if in doubt.⁴

NAEAC believes that the problem of relief of animal suffering merits higher priority in scientific enquiry.

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² Pain and Suffering in Experimental Animals in the United Kingdom, RSPCA, West Sussex, 1985, 12
³ Ethics in Animal Experimentation, June 1986
APPENDIX

ANIMAL CARE/VETERINARY NURSING COURSES

- Bay of Plenty Polytechnic
- Christchurch Polytechnic Institute of Technology
- Eastern Institute of Technology
- Massey University
- Nelson Marlborough Institute of Technology (Nelson)
- Otago Polytechnic
- Southern Institute of Technology (Invercargill)
- Universal College of Learning
- Unitec (Auckland)
- Vet Nurse Plus
- Waikato Institute of Technology
- Wanganui Veterinary Services
- Weltec
## Animal Welfare Score Sheet

(Reprinted with permission from the University of Otago)

One sheet per animal to record parameters listed below

<table>
<thead>
<tr>
<th>Animal/Species #</th>
<th>Pre-study Bodyweight</th>
<th>Date of Treatment/Operation:</th>
<th>Surgeon:</th>
<th>AEC #:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Date</td>
<td>Day</td>
<td></td>
</tr>
</tbody>
</table>

### Body Weight & B.A.R. Score

- Weight yesterday
- Weight today
- Weight change
- BAR (bright, alert, responsive)
- Approach response
- Inquisitive behaviour

### General Clinical Signs

- Inactive
- Hunched posture
- Coat rough, fur on end
- Red eye/nose discharges
- Pink staining of neck
- Dehydration – Skin turgor test

### Behavioural Signs of Pain in Rats

- Back arch
- Hunched up with arched back
- Belly press
- Presses belly to cage floor
- Writhe
- Twisting of body or flank
- Stagger
- Sudden loss of balance/gait
- Twitch
- Sudden spasm of flank muscles
- Fall
- Rat falls over

### Water Balance

(Healthy animals drink approx 10% body weight per day, e.g. a 300gm rat should drink 30mls every 24 hours)

- Start weight of bottle (A)
- Current weight of bottle (B)
- Water intake (A-B) mls

### Operation Site

- Wound OK
- Bleeding
- Sutures/Clips OK

### Post-Op Support – Analgesic Administration

- Drug
- Dose
- Fluids by SC injection
- Other drugs

### Signature
How to use the Animal Welfare Score Sheet

Humane End-points:

1. Weight loss of 10 percent or more over 24 hours
2. Weight loss of 20 percent or more plus one other clinical sign compared with control group
3. Weight loss of 25 percent compared with control group

Key Points:
- One score sheet per animal
- The 12 boxes across the page can be used for 12 different time points.
- For critical post-op cases, animals should be checked every 4 hours (three times per day).
- It is recommended that a pre-operative examination be made of the animal before anaesthesia, to observe the animal. This is time zero in the first box.
- Look for evidence of red discharge from nose or eyes (a non-specific sign of stress).
- Look at the coat, it should be groomed smooth and shiny. A rough coat has fur standing on end, because the animal is too sick to groom itself.
- Look for dehydration – if the skin can be gently pulled away from the body and remains that way, the animal is significantly dehydrated.
- Look at the colour of the ears and feet. They should be pink.
- Look at the behaviour of the animal. It should be B A R.
- Record the weight of the water bottle before surgery.

Use the form to record animal welfare. Score normal animal as 0; score 1, 2, 3 for in severity
- Changes in physical condition with time, after surgery. When several animals are used simultaneously, it is difficult to remember how any individual animal looked the previous day.
- Administration of analgesic pain medication.
- Administration of subcutaneous fluids.
- Condition of the surgical sites, look for blood or other discharges.
- Fluid drunk by the animal. This is one of the best indicators of wellbeing. Animals in pain do not drink much. Healthy animals normally drink approximately 10 percent body weight per day. Hence a 300gm rat should drink 30mls every 24 hours.
- Weigh the water bottle and difference gives approximate volume consumed. If the animal is not drinking 10 percent of body weight per 24 hours, it is probably in pain. Pain medication should be increased and additional fluid administered by subcutaneous injection. Seek veterinary advice on how to do this.
- Body weight of the animal. Weight should be maintained. Significant weight loss is used as a humane endpoint.

Signs of pain in the rat are well described:
- Back arch, belly press, writhe, stagger, twitch and fall.
- Careful observation is needed because the behavioural sign may be subtle or occur very quickly.

Skin turgor test: pull up the skin; if it remains tented, then the animal is dehydrated. See arrow at left
• Observation of animals several times a day may be needed in order to detect these signs.
• When you observe one of these signs score it as a ✓ each time.
• The observation of any of these signs is an important clinical finding – it indicates the animal is in pain and additional analgesia is needed. Contact the vet for assistance.