DEPARTMENT OF
PRIMARY INDUSTRIES

Code of Practice for the Housing and Care of Laboratory Mice, Rats, Guinea Pigs and Rabbits

Victoria
The Place To Be

Acknowledgements

"The Bureau of Animal Welfare would like to acknowledge the input of the Code writing group, and Monash University for the provision of a number of example monitoring appendices. Cover photography courtesy of Monash University, University of Melbourne and Walter Eliza Hall Institute"

Code of Practice for the Housing and Care of Laboratory Mice, Rats, Guinea Pigs and Rabbits.

Published by the Victorian Government Department of Primary Industries
475-485 Mickleham Road
Attwood Victoria 3049 Australia
Telephone: +61 3 9217 4200
Facsimile: +61 3 9217 4299

Also published on http://www.dpi.vic.gov.au/animalwelfare

Approved by the Governor in Council 12 October 2004.

Issued by the Minister of Agriculture *Prevention of Cruelty to Animal Act 1986*
Published in the Victorian Government Gazette, 16 December 2004

Authorised by the Victorian Government
1 Spring Street
Melbourne Victoria 3000 Australia

ISBN 1 74146 288 6

For further information, visit the DPI Website at http://www.dpi.vic.gov.au
or contact the DPI Customer Service Centre on 136 186

Code of Practice for the Housing and Care of Laboratory Mice, Rats, Guinea Pigs and Rabbits

Department of Primary Industries

Code of Practice for the Housing and Care of Laboratory Mice, Rats, Guinea Pigs and Rabbits

Contents page

1. Introduction, Purpose and Scope of this Code 7

2. Definitions and Acronyms 8

3. Principles, Minimum Standards and Recommendations for the Housing and Care of Laboratory Mice, Rats, Guinea Pigs and Rabbits 10

3.1 Nutrition 10
- 3.1.1 Food 13
- 3.1.2 Water 14

3.2 Animal Enclosures 15
- 3.2.1 Materials and design 17
- 3.2.2 Space requirements 17
- 3.2.3 Social requirements 19
- 3.2.4 Bedding and nesting 19
- 3.2.5 Special requirements 20

3.3 Climate Control 20
- 3.3.1 Temperature 21
- 3.3.2 Relative humidity 23
- 3.3.3 Ventilation and air quality 23
- 3.3.4 Noise and vibration 26
- 3.3.5 Light 27
- 3.3.6 Emergency plans and alarm systems 28

3.4 Behaviour and Environmental Enrichment 28
- 3.4.1 Behaviour 29
- 3.4.2 Environmental enrichment and complexity 29

3.5 Maintenance and Hygiene 32

3.6 Handling and Basic Procedures 33

3.7 Health Monitoring 35

3.8 Transportation 36

3.9 Euthanasia 37

3.10 Monitoring and Records 39

4. Further Information Sources 41

Contents page continue.

Appendices

Appendix 1	Minimum standards for housing of laboratory mice, rats, guinea pigs and rabbits	44
Appendix 2	Methods of euthanasia for post-neonatal laboratory mice, rats, guinea pigs and rabbits	47
Appendix 3	Guidelines for euthanasia of fetal and neonatal mice, rats, guinea pigs and rabbits	50
Appendix 4	Example Animal monitoring sheet	52
Appendix 5	Example Welfare assessment score and judgement sheet	56
Appendix 6	Example Monitoring sheet for animals after surgery or invasive procedures	58
Appendix 7	Example Daily breeding record sheet	60
Appendix 8	Example Monthly animal record sheet	62
Appendix 9	Example Numerical score sheet for neonatal genetically modified rodents	64
Appendix 10	Example Phenotype report for genetically modified and/or cloned animals	66

1. Introduction, Purpose and Scope of this Code

The 'Code of Practice for Housing and Care of Laboratory Mice, Rats, Guinea Pigs and Rabbits' applies throughout Victoria. It establishes minimum standards for the housing and care of these animals under The Prevention of Cruelty to Animals Act 1986 (The Act) and Regulations 1997. This code is intended to be read in conjunction with the current *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (The Australian Code).*

Minimum standards in this code will be enforceable, as it is a mandatory 'code of practice' under Part 3 of The Act. Proposed variations to these standards, as part of an experimental or breeding protocol, must be justified to and approved by an Animal Ethics Committee.

The recommendations serve to provide further guidance and should be promoted wherever possible to achieve practice above and beyond the minimum standards.

This document has been developed in collaboration with a number of laboratory animal veterinarians, animal technicians, animal house managers, inspectors and animal welfare officers from academic and research institutions and private industry. This consultation ensured that wide experience and specialised knowledge relevant to this animal industry in Victoria formed the basis of the information presented herein. Relevant international codes, guidelines, standards and references were also considered as were submissions from the Victorian Animal Welfare Advisory Committee and a period of public comment. This is the first code of its type and content in Australia.

It should be noted that this code is not intended as an exhaustive source of information on the housing and care of the four species covered. For example, details of dietary requirements, structural requirements of animal rooms and animal handling and basic procedures are not included as it was felt that an overly prescriptive document would not best serve the wide variety of institutions and situations in which it has relevance.

Appendices 4-10 provide examples of various monitoring sheets that are currently used in some institutions and may be recommended to assist in monitoring and recording in institutions in general.

It is intended that this code will assist a wide spectrum of people involved in the use of animals in scientific procedures to ensure that minimum standards of animal care, housing and welfare are achieved and that recommendations of 'best practice' and the 'three Rs' of Russell and Birch - Replacement, Reduction and Refinement - are promoted wherever possible. As new information on how best to house and care for these laboratory animals becomes available, the code will be updated and reviewed by the Bureau of Animal Welfare.

2. Definitions

- **Abnormal Stereotypy:** A repeated sequence of movements, which has no obvious function.
- **Animal Ethics Committee (AEC):** A committee, the principal function of which is to determine the ethical and animal welfare practices that are to apply to the carrying out of scientific procedures. This committee must be constituted in accord with the terms of references and membership laid down in *The Australian Code.*
- **Animal welfare:** An animal's quality of life based on an assessment of an animal's physical and psychological state as an indication of how the animal is coping with its circumstances.
- **Best practice:** Best practice is that which is agreed at a particular time, following consideration of scientific information and accumulated experience. It is a higher standard of practice than the minimum standard.
- **Clone:** A genetic copy of another living or dead animal. It is not a twin derived from the fertilisation of an egg by a sperm.
- **Euthanasia:** the humane killing of an animal, in the interests of its own welfare, to alleviate pain and distress (see humane killing).
- **Genetic modification (of an animal):** the use of any technique for the modification of genes or other genetic material, but not including the use of natural processes such as sexual reproduction.
- **Genotype:** The genetic constitution of an individual.
- **Humane killing:** the process of killing an animal with minimal pain and distress (see Euthanasia).
- **Investigator or teacher:** Any person who uses animals for scientific procedures.
- **Microbiological Barrier:** a combination of animal handling procedures and housing which enables the animals' microbiology to remain constant over time (ie. biocontainment or bioexclusion).
- **Phenotype:** Appearance and behaviour of an organism resulting from interaction between its genome and its environment.
- **Project:** A single program of scientific procedures as defined under Section 25 of The Act.
- **Standard Operating Procedure (SOP):** Detailed description of a standardised procedure.
- **The Act:** The Prevention of Cruelty to Animals Act 1986.
- **The Australian Code:** The Australian Code for the Care and Use of Animals for Scientific Purposes (current edition).

Acronyms

- **AEC:** Animal Ethics Committee.
- **ACH:** Air changes per hour
- **ANZCCART:** Australian and New Zealand Council for the Care of Animals in Research and Teaching.
- **ANZSLAS:** Australian and New Zealand Society for Laboratory Animal Science.
- **AQIS:** Australian Quarantine Inspection Service.
- **AVA:** Australian Veterinary Association.
- **IATA:** International Air Transport Association.
- **IBC:** Institutional Biosecurity Committee
- **IVCs:** Individually Ventilated Cages (ie. microisolators)
- **NHMRC:** National Health and Medical Research Council.
- **OGTR:** Office of the Gene Technology Regulator.
- **PC:** Physical Containment (level).

3. Principles, Minimum Standards and Recommendations for the Housing and Care of Laboratory Mice, Rats, Guinea Pigs and Rabbits

Mice and Rats

The laboratory mouse and rat are derived from a largely nocturnal burrowing and climbing ancestor who favoured building nests for temperature regulation and reproduction. As such, they retain many of the traits of their wild counterparts, for example, grooming, exploratory activity, searching for food, burrowing, climbing and gnawing. Housing systems should aim to encompass these behavioural and physiological needs.

These animals have relatively poorly developed vision, but highly developed senses of smell and hearing. Rats, in particular, are very sensitive to ultrasound. Laboratory rats and mice are very social animals, and as such, disruption to groups should be minimised. Young rats, in particular are very exploratory and active, and interact socially to an enormous degree.

Guinea pigs

Guinea pigs may appear nervous but are tame and can be handled easily. Vocalisation appears to play an important part in guinea pig social and sexual behaviour, and they often call for attention from animal care staff. They naturally thrive in family or weaner groups, and although male guinea pigs may fight, aggression between sexes is uncommon. Guinea pigs can be housed for breeding in pairs or harems and the young are fully developed at birth. Weaning takes place at 2-3 weeks, but generally the young are eating solid food and water within a few days of birth.

Rabbits

Comparative studies of domesticated rabbits living in groups in large enclosures have shown that they retain a wide behavioural repertoire, similar to their wild ancestors. There is increasing evidence to show that rabbits denied the freedom of natural behaviour and exercise can lose normal locomotor activity and suffer skeletal abnormalities. Within the practicalities of laboratory housing of rabbits, an environment adequate to allow performance of a wide behavioural repertoire should be provided. The rabbit is a naturally gregarious species so attention should be paid to their social wellbeing. These requirements are preferably met by housing rabbits in pens.

3.1 Nutrition

3.1.1 Food

All four species practice coprophagy; the ingestion of a special faecal pellet coated in mucus. They are cautious feeders, often avoiding unfamiliar foods. These animals have constantly erupting teeth and interference to wear will lead to malocclusion, which causes difficulty with eating and swallowing.

Guinea pigs are unable to synthesise vitamin C (ascorbic acid) in sufficient quantity to meet their daily requirements. Insufficient Vitamin C intake will lead to debilitation, increased susceptibility to disease and eventually to scurvy. Guinea pigs, in particular, do not like change in their diet.

Minimum Standards for Nutrition:

(i) Laboratory animals must receive a palatable diet, which is free from contaminants and provides the nutritional requirements appropriate to the species, age and breeding stage of the animal.

(ii) Food must be stored in cool, vermin-proof rooms under conditions that prevent it from becoming a health risk to the animals.

(iii) Communication between investigators and animal house staff must be maintained concerning any supplementation or manipulation of diets.

(iv) Diets for guinea pigs must fulfil their vitamin C and E requirements.

(v) Consideration must be given to the type of presentation of food when feeding young or handicapped animals.

General Recommendations:

(i) In the selection, production and preparation of food, precautions should be taken to avoid chemical, physical and microbial contamination to ensure that food is safe for the animals and their young. All food hoppers and utensils should be cleaned regularly and sterilised when necessary. All fruit and vegetable supplements should be appropriately washed prior to presentation to the animals.

(ii) It is recommended that feeding of young or handicapped animals (for example; those post-surgery or with muscular dystrophy) be facilitated by provision of food in a Petrie dish or hand-feeding as necessary.

(iii) When moist food is used, it should be replaced regularly to ensure palatability and food safety.

(iv) The animal house manager or a laboratory animal veterinarian should be consulted prior to supplementation or manipulation of complete commercial diets.

(v) Consideration should be given to the rotation of use and storage of formulated diets such that they are used within the recommendations of the manufacturer (particularly formulated diets supplemented with Vitamin C).

(vi) Food used in microbe-controlled environments is often autoclaved to avoid the introduction of food-borne pathogens. As autoclaving decreases the concentrations

of some vitamins and antioxidants, diets should be based on formulations that contain higher concentrations of heat-labile ingredients.

(vii) Where animals are held in groups, care should be taken to ensure that subordinate animals have sufficient access to food and water. It is recommended that more than one access point for food (and water) be provided to reduce the possibility of aggressive competition.

(viii) Any significant changes in food intake should be investigated.

Species Specific Recommendations:

Mice:

(i) Consideration should be given to the presentation of food for certain animals that have abnormalities of the teeth and jaw.

Guinea Pigs:

(i) Taking into consideration autoclaving or irradiation requirements, Vitamin C can be supplied in the pelleted ration (800mg/kg finished diet) or supplemented in the drinking water (1g/litre), prepared fresh daily. Fresh vegetables can also be used to provide Vitamin C and should be thoroughly washed prior to presentation.

(ii) Guinea pigs have a high dietary fibre requirement (16%) which is best met by supplying them with good quality lucerne hay. They should be provided with a diet of 20% minimal protein.

(iii) The feed should be appropriately stored to maintain active levels of Vitamin C. As a guide, one half of the Vitamin C may be lost 90 days after the diet has been commercially mixed and stored above 22ºC.

(iv) Guinea pigs are susceptible to anorexia following experimental procedures and may require special attention to resume eating. The use of a pellet mash mixed with water and hand fed to guinea pigs will often be sufficient for normal appetite to be resumed. A faecal pellet can be included to restore microbiological activity in the digestive tract after periods of anorexia.

(v) Consideration should be given to the type or placement of the feed hopper outside the cage to control wastage of food and contamination of the feed with faeces.

Rabbits:

(i) It is recommended that foods such as hay, fruits, vegetables, legumes or green feeds be fed to supplement commercial pellets and to reduce the monotony of a fixed ration diet.

(ii) A high fibre diet should be provided to help prevent diarrhoea and hairballs. A diet with between 18-25% fibre is recommended.

(iii) If a restricted diet is required to be fed to rabbits, it should be provided at routine times.

3.1.2 Water

Minimum Standards for Water Provisions:

(i) Potable water must be available to all animals at all times.

(ii) Precautions must be taken to avoid flooding in solid-bottomed cages.

(iii) If Vitamin C is to be provided at effective levels in the water for guinea pigs, a non-copper delivery system and daily preparation of the water must be used.

General Recommendations:

(i) The source and method of water supply should minimise microbial and chemical contamination.

(ii) Under certain conditions of transport, water should be provided in the form of a moist diet (see Section 3.8).

(iii) Water bottles or containers should be sanitised or sterilised. They should be sufficiently transparent to enable water availability to be easily checked and have a wide mouth to facilitate cleaning. Water bottles or containers should always be replaced with clean, freshly filled ones and should not be able to easily tip or spill.

(iv) Automatic watering systems should be serviced and cleaned regularly to avoid malfunction and the risk of spread of infection, drowning or drought. This should include checking for the correct pressure in the drinking valves to prevent back-flow of water into the lines when animals drink from, or play with, the valve. The nipples should be located at a suitable height to enable access for all animals. Some animals need to be taught to use automatic watering devices.

(v) These species are very susceptible to water deprivation. Water supply should be checked daily and if a problem is confined to one cage/box, blockage of a watering nipple or bottle should be considered as a cause in the first instance.

Species Specific Recommendations:

Guinea Pigs:

(i) These animals often waste water by playing with the sipper and therefore automatic watering systems are often not used. The sipper tube should be located outside the cage to prevent excessive wetting. If automatic watering devices are used, some animals require training.

Rabbits:

(i) Open watering systems should be avoided to prevent infection of the dewlap.

3.2 Animal Enclosures

The design of animal housing should facilitate well being of the animals, meet research requirements, minimise experimental variables, and isolate the animals from wide variations in temperature and humidity and from vibration and sources of loud noise. For detailed requirements of the design of animal rooms, refer to *'Housing for Laboratory Rats, Mice, Guinea Pigs and Rabbits'*, A.L. Hargreaves, ANZCCART 2000. Consideration should also be given to occupational health and safety requirements when designing animal housing.

The formation of social groups, and utilisation of enrichment devices and bedding material to enable climbing, burrowing, nesting, reproduction and thermoregulation are as important as provision of adequate cage space for these species. Mice, rats and guinea pigs tend to avoid open spaces.

Mice:
Mice, in particular, like to compartmentalise their behaviours and often use urine marking and bedding to assist.

Rats:
Rats enjoy standing on their hind legs and peering from their enclosures. They are excellent climbers, utilising the full height of their housing, and also use urine spotting as a territorial marker.

Guinea Pigs:
Guinea pigs are timid, social animals and can be slow to adapt to change in their environment. As these animals have a poorly developed capability for either jumping or climbing, they may be housed in a relatively low walled, open topped pen or cage. Natural behaviours include stampeding which may be avoided by providing sheltered or hiding places, and avoiding sudden noise, disturbance or overcrowding.

Rabbits:
There is a need to provide enough space to permit rabbits to meet their species-specific needs. This includes sufficient exercise for skeletal development (hopping, rearing up etc) and direct social contact with other compatible rabbits. Rabbits can be housed in cages or floor pens. Pens are enclosures that allow for greater freedom of movement and expression of social behaviours. Cages are fully enclosed containers that may restrict freedom of movement and social interaction because of their size and possibly design. The advantages and disadvantages of housing rabbits in cages versus pens are discussed comprehensively in the *'Guidelines for the Housing of Rabbits in Scientific Institutions'* developed by the NSW Animal Research Review Panel (see Section 4). These guidelines recommend the use of pens for housing laboratory rabbits.

Minimum Standards for Animal Enclosures in General:

(i) Animal enclosures (cage or pen) must meet or exceed the minimal space requirements outlined in this Code to permit reasonable freedom of movement and normal postural adjustments.

(ii) Enclosures must be compatible with what is known of the behavioural and physiological needs of the animals.

(iii) Enclosures must be durable and provide a comfortable environment, be maintained in good repair, be kept clean and be escape-proof.

(iv) Enclosures must confine animals safely with easy access to food, water and ventilation and enable easy monitoring and access to the animals.

General Recommendations:

(i) Size, design and materials used in the construction of animal enclosures may affect many of the environmental factors normally controlled at room level and thus may affect the characteristics of the microenvironment. Refer to Section 3.3 for recommendations concerning climate control.

(ii) Special containment facilities are required for the use of radioisotopes, infectious agents and highly toxic substances. For specific requirements and recommendations, refer to appropriate guidelines.

3.2.1 Materials and Design of Animal Enclosures

The different materials used for animal enclosures affect shading and social contact via degree of transparency, as well as heat and noise conduction. Although mesh or wire floor cages may offer some advantages over solid floor cages, for example to reduce disturbance during cleaning and to reduce the risk of cage flooding, solid floors provide a more comfortable and insulated surface with a reduction of the risk of injury. Faulty mesh or wire floors and lids can lead to serious injuries.

The design of housing, whether enclosed, such as 'shoebox-style' cages, or open-type designs such as pens, determine the air movement and rate of dissipation of heat. Filter tops exacerbate the effects on ventilation of an enclosed design. They raise the temperature and relative humidity inside the cage and hasten the build-up of carbon dioxide and ammonia.

Minimum Standards for Materials and Design of Animal Enclosures:

(i) Enclosures must be designed and made of materials that are comfortable and safe for the animals, and withstand cleaning agents and techniques.

(ii) Housing must enable easy monitoring of the animals.

(iii) Nesting boxes (or equivalent) must be provided for breeding animals.

(iv) Wire or mesh floors and lids must be carefully selected and maintained to minimise the risk of foot and leg injuries. A solid mat or suitable substrate must be provided.

(v) Insect vectors of myxomatosis and calicivirus rabbit disease (ie. flies and mosquitos) must be prevented from entering rabbit enclosures.

General Recommendations:

(i) Ventilated racks and IVCs used to house laboratory rodents provide separation at the rack and cage level, respectively. Consideration should be given to the additional heat load, noise and draughts provided by these systems. Separation of the motorised components of such systems reduces noise and heat impacts.

(ii) In the design of animal enclosures, consideration should be given to make the housing environment suitably complex or enriched (see also Section 3.4).

Species Specific Recommendations:

Mice:

(i) Wire lids for mouse cages should be carefully selected to prevent toe injuries

Rats:

(i) Galvanised metal should be avoided in the long term for rats, due to excessive risk of zinc toxicity.

Rabbits:

(i) The *'Guidelines for the Housing of Rabbits in Scientific Institutions'* (see Section 4.) is recommended for principles of rabbit pen design. Provision for rabbits to withdraw from others should be provided, and subdivision to facilitate cleaning and catching animals in pens is recommended.

3.2.2 Space requirements.

For all species, it is recognised that social relationships, enclosure shape and internal furnishings may be as important to the animal as overall size of the enclosure. The shape of the cage or floor pen may contribute to the security and comfort of the animals.

Guinea Pigs:
Guinea pigs prefer to lie down and stretch out and also to congregate around the periphery of the enclosure. An elongated shape maximises the length of space and wall for the occupants and reduces the central more exposed space. Guinea pigs do not readily climb, and can be housed in open topped pens or cages. They do sometimes stand up and should be provided with adequate height to do so.

Rabbits:
Rabbits like to lie down outstretched. They often stand upright, hop or play, which is essential for normal skeletal and social development.

Minimum Standards for Space Requirements:

(i) Adequate space must be provided to allow animals to exercise, to maintain the social stability of the group and to perform normal physiological and behavioural activities.

(ii) Stocking densities must be adjusted for different breeds, ages and growth of the animals.

(iii) The details of space requirements are given in Appendix 1, where the stated dimensions refer to internal measurements of the animal enclosure. Animal housing must comply with these dimensions with the exception of short-term housing of animals post-weaning and prior to issue, provided there are no associated deficits in their welfare.

(iv) All four species either climb or assume upright posture at times, which must be accommodated without hindrance by the height of the enclosure.

3.2.3 Social requirements.

The way in which the cage or pen is stocked has direct social and welfare consequences for the animals. Single housing will cause social deprivation and impacts on the animal's capacity to thermoregulate.

Intense territoriality may be seen in reproductively active male animals. Castration prior to puberty may prevent aggression and fighting. Pregnant and lactating females may prove aggressive in nest defence. Some strains of rodents are more aggressive than others, which results in fighting within groups.

Minimum Standard for Social Requirements:

(i) Animals must be housed in social groups unless the welfare of the animal would be compromised by group housing.

General Recommendations:

(i) Stocking density should permit animals in a group to disperse or withdraw comfortably and establish natural hierarchies within the group. It is then recommended to keep the composition of the group stable.

(ii) The composition of the group with regard to sex, age, temperament and familiarity should be considered when housing animals. Animals displaying aggression toward one another should be separated.

(iii) Where single housing is justified on welfare grounds or as part of an experimental protocol, consideration should be given to the provision of environmental enrichment and social contact through visual and auditory contact, wherever possible. This will help to avoid isolation stress, which may result in increases in nervousness, aggression, and susceptibility to convulsions, certain drugs and metabolic and adrenocortical activity.

Species Specific Recommendations:

Mice and Rats:

(i) Wean animals into social groups to prevent fighting and to create stable, manageable hierarchies.

Guinea Pigs:

(i) Guinea pigs are social animals and prefer to live in groups of 5-10 animals. They should be kept in compatible groups or breeding pairs or harems. Breeding groups of 3-10 males and 15-30 females can also be managed, by allowing a social group to increase naturally thus evolving a social hierarchy or by adding new females. Females can usually be housed together even if strangers. Males can be kept in groups up to 4 months and then need to be in pairs. Adult males can fight, especially in the presence of an oestrus female.

Rabbits:

(i) Group housing should be provided for rabbits. It should include the opportunity for the animals to rest and withdraw from each other.

(ii) Wherever possible littermates should be housed in groups post-weaning.

(iii) Uncastrated mature male rabbits (ie. over 12-14 weeks of age) should not be housed together. Castration prior to puberty (ie. before 12-14 weeks) is recommended, where appropriate, to prevent intolerance and fighting amongst sexually mature males.

(iv) Rabbits that cannot be housed in groups (eg destabilised hierarchies, intact males or those involved in AEC approved experimental protocols) should have extensive olfactory and visual contact with other rabbits, as well as the ability to withdraw.

3.2.4 Bedding and nesting.

Minimum Standards for Bedding and Nesting:

(i) Bedding material appropriate to the species must be provided in animal enclosures with solid flooring.

(ii) Nesting material must be provided for breeding animals.

(iii) The nesting area for rabbits must be designed to allow the doe to exhibit normal nesting behaviour and contain the young rabbits in the early post-partum period, with sufficient size to permit suckling. In addition, breeding does must be provided with adequate nesting material at least five days before parturition until the litter is weaned.

(iv) Bedding and nesting materials must be comfortable and safe for the young and adults, dry, absorbent, dust-free, low-allergenic, non-toxic, non-injurious and free from vermin and other contaminants.

Species Specific Recommendations:

Mice and Rats:

(i) Materials likely to entangle rats and mice should not be used as permanent bedding.

Guinea Pigs:

(i) Guinea pigs do not build nests but require some form of bedding to provide cover for young and burrowing for general environmental comfort and enrichment. Recommended examples of suitable bedding material for guinea pigs include low-dust wood shavings (not sawdust as this may adhere to the preputial area of males), shredded paper or hay.

Rabbits:

(i) Recommended types of bedding or nesting material for rabbits that may be used alone or in combination include straw, shredded paper and non-toxic wood.

3.2.5 Special Requirements of Animal Enclosures

Minimum Standards for Special Requirements of Animal Enclosures:

(i) Animal house personnel and investigators must be aware of any special requirements of animals involved in experimental protocols, which must be documented in the experimental protocol. Such animals must be identified and records kept of close monitoring for signs of distress or discomfort, particularly if they are transferred between facilities with different housing conditions.

(ii) The special care and housing requirements of albino, genetically modified, cloned, aged, immunocompromised animals must be provided. Similarly, animals that have been affected by disease, surgery or pharmacological compounds may have particular social, dietary or behavioural characteristics that must be considered.

(iii) Where microbiological barrier conditions are in place for biocontainment or bioexclusion, steps must be taken to provide a complex cage environment and to allow interactions with animal care staff to meet the needs of the animals.

Mice and rats:

(i) In addition to a lack of fur for thermoregulation, nude mice and rats do not have eyelashes for protection of the eye and eyes and skin can easily become irritated by bedding fibre. It is recommended that low-dust bedding and nesting material be used for these strains.

3.3 Climate Control

The climate experienced by the laboratory animal is that of the 'microenvironment' in its enclosure. Except where animals are housed in ventilated rack systems or IVCs, climatic variables are generally set and controlled for the room or 'macroenvironment' in which the animal enclosures are located. Controlling the microenvironment through the macroenvironment requires an understanding of how the components of climate are affected by the type of animal enclosure.

The design of the animal enclosure, the materials used in its construction, the type of bedding and the stocking density all affect the climate of the microenvironment and consequently the welfare of the animals. In addition, variations in any one of these parameters may impact on the experimental results as well as the working conditions for personnel.

Monitoring and recording the conditions inside the macro- and microenvironments is essential to good climate control. Emergency plans should be in place, including mechanisms to alert appropriate personnel in the event of power failure or unacceptable changes in climatic variables.

Laboratory mice and rats generally choose to manipulate their own microenvironments via activities such as huddling, nest building, tunnelling and burrowing. In general, the rodent's ability to control temperature, humidity and lighting is as important to its welfare as specifying ambient conditions within the room.

3.3.1 Temperature

Temperature and humidity should be considered together due to their close interrelationship. Animals show a graded thermal and adrenal stress response to increasing *effective temperature* (an index of animal comfort), regardless of actual temperature.

Neonates have no autonomous thermoregulation in the first week, but their appropriate thermoneutral zone is 30-34°C. This is normally achieved within the microenvironment of a well structured nest. Lactating females have a higher metabolic rate and show a preference for lower temperatures. Old, sick, nude, immunocompromised or experimentally stressed animals generally have poor homeostasis. Variability in ambient temperatures is more likely to result in changes in their body temperature, resulting in further stress.

Uniformity of temperature throughout a room will depend on the effectiveness of ventilation and the positioning and material of cages or boxes in a racking system. There is an effect due to heat conduction within a rack of rodent cages from the body heat of the animals - the cages at the top and middle may be up to 5°C hotter than the bottom cages.

Guinea Pigs:
In general, this species is better able to withstand cold than heat, if provided with sufficient bedding and protection from draughts. Reproductive rates will decline significantly if room temperatures are above 25ºC for any length of time. Pregnant sows are susceptible to heat stress at higher temperatures (ie > 30ºC) and survival of young is greatly reduced at 17ºC.

Rabbits:
Low temperatures are fairly well tolerated by rabbits but heat and drafts are not well tolerated. Temperatures above 30°C, combined with high relative humidity, can cause heat stress, which may result in infertility or mortality.

Minimum Standards for Room Temperature:

(i) Room temperatures must be measured and recorded once daily and maximum and minimum values must be recorded wherever possible.

(ii) Room temperature must be maintained within the temperature range specified for each species (see Appendix 1).

(iii) When housing very aged, very young or hairless animals, or animals with a reduced thermoregulatory capacity as the result of genotype or an experimental protocol, higher room temperatures than those indicated in Appendix 1 may be required. Suitable bedding material or thermal heat pads must be provided for such animals.

General Recommendations:

(i) Laboratory animals are very susceptible to sudden fluctuations in temperature and these should be avoided wherever possible.

(ii) In setting the temperature of the animal room, consideration must be given to the potential impact of sunlight, the heat generated by animals, and the rate at which heat is dissipated from cages. For example, open, metal or wire cages lose heat more quickly than plastic ones, and more animals generate more heat.

(iii) The design and furnishing of the animal enclosure should be conducive to assisting the animals to thermoregulate. The animal enclosure should enable group-housed animals to warm themselves by huddling together or by using bedding or nesting material and also provide sufficient space for animals to disperse to increase heat loss.

(iv) Temperature should be continually monitored, and an optimum range thermostat set. It is desirable that monitored room temperatures are centrally displayed in the animal house. In addition, the temperature in a representative range (ie. top, bottom and middle locations in a rack) of the smallest unit of animal housing should be monitored periodically. Note: the temperature in the individual cage may be up to 5°C higher than the room temperature, depending on the stocking density and position of the enclosure in the room.

Species Specific Recommendations:

Rabbits:

(i) It is recommended that room temperature be maintained within 15-24°C.

3.3.2 Relative humidity

Owing to the inter-relationship between humidity and temperature, at a given temperature higher relative humidity causes an increase in *effective temperature*. Room ventilation and stocking rate of the enclosure impact on relative humidity as the respiration of animals and evaporation from excreta generate moisture inside the enclosure.

Humidity variations are less significant than temperature variations. Low relative humidity causes higher dust levels, increased levels of respiratory infections and possible skin lesions. High relative humidity increases thermal stress and ammonia levels, and lowers resistance to infection.

Minimum Standards for Relative Humidity:

(i) Relative humidity in enclosures must be kept within the range of 40 to 70% wherever possible.

General Recommendations:

(i) Enclosed tops, such as filter tops or cage bonnets, can significantly impede airflow, trap moisture inside the cage and raise humidity. Allowances should be made accordingly.

(ii) Room humidity should be monitored and kept well below 70% to ensure that the relative humidity of the enclosure stays within the acceptable range. Refer to figures in italics and brackets in Appendix 1 for the species specific recommended levels of relative humidity in the animal enclosure.

3.3.3 Ventilation and Air Quality

Ventilation regulates temperature and humidity, controls air quality and facilitates the movement of air between the macro- and micro- environments of the room. Like temperature and humidity, ventilation is usually controlled at the room level, but it is the conditions at the level of the animal enclosure that are important. These, in turn, are affected by the size and thermal load of the room and the stocking density and design of the animal enclosures. Wire grid-floored cages/boxes have approximately 90% of the room ventilation rate, while solid floored enclosures have approximately 60% of the room ventilation rate. Filter-tops on enclosures markedly restrict air exchange and can increase ammonia levels by 50-100%.

The most common gaseous contaminant in animal facilities is ammonia resulting from the decomposition of nitrogenous waste in excreta. Poor ventilation, increases in relative humidity and poor hygiene all contribute to elevated concentrations of ammonia which can irritate the respiratory tract and increase the susceptibility of animals to respiratory disease.

Ammonia may exceed 25ppm in rodent cages when bedding changes and cleaning are due. At this time, the room ammonia level may be less than 10ppm. Ammonia above 25ppm inside rodent cages is a potent co-irritant and can act synergistically with respiratory pathogens. Rabbits are even more sensitive to ammonia build-up.

Sealed IVCs are potentially dangerous, as animals may die rapidly if the ventilation fails and there is nobody to intervene. In sealed IVCs where ventilation fails, carbon dioxide can rise above 30 000 ppm (3%) in under 30 minutes, and above 50 000 ppm (5%) in little over an hour. Unrectified ventilation failure over a couple of hours is likely to be fatal.

Minimum Standards for Ventilation and Air Quality:

(i) Fully operational rooms or IVCs for laboratory animals must be provided with draught-free, fresh or conditioned air distributed continually and throughout.

(ii) Average concentrations of ammonia in *animal rooms* must not exceed 25ppm over an 8 hour day, which is also the upper limit for human occupational health.

(iii) Average concentrations of ammonia *in the smallest unit of animal housing* must not exceed 25ppm.

(iv) Recirculating ventilation systems must be regularly serviced.

General Recommendations:

(i) Consideration should be given to the two components of ventilation; air speed and air movement, which cover the number of air changes per hour, and air quality.

(ii) Ventilation should be sufficient to prevent the build-up of noxious carbon dioxide, ammonia, humidity, dust and infectious agents. While 10 to 20 room ACH may be adequate for conventional animal rooms, this rate does not guarantee that ventilation will be adequate at the enclosure level, particularly if filter tops are used.

(iii) Concentrations of carbon dioxide in IVCs should be less than 5000ppm (0.5%). Refer to Section 4 for available air quality monitoring devices.

(iv) Bedding or nesting material should be considered in conjunction with ventilation as its absorptive properties can decrease the production of ammonia. Refer to (vii) and (viii) regarding the measurement of ammonia levels.

(v) The air distribution system should be configured to maximise energy efficiency and deliver as even a proportion of air as possible to each animal enclosure. Careful attention should be given to inlet and outlet positions to ensure good air circulation and avoid draughts and noise.

(vi) Ventilation systems should be set at differential air pressures within a building to meet the different requirements of 'barrier systems', such as those used in PC3, PC4 and SPF conditions. For example, higher pressures should be used in clean areas relative to dirty or biohazardous ones, in order to minimise contamination. In addition, germ-free or defined flora populations, SPF breeding facilities, and colonies of aged, immunocompromised animals or those involved in disease models require a higher level of control of the microbial environment than that used in conventional housing. (See Sections 3.2.5 and 3.7)

(vii) To prevent excessive levels of ammonia in animal enclosures, consideration should be given to reducing stocking densities, open versus closed shelving, frequent cleaning and avoiding the use of filter top cages.

(viii) Humans can smell ammonia at a concentration as low as 8ppm and any smell of ammonia should be investigated. The concentration of ammonia should be monitored using one of the available gaseous detection devices placed in the animal enclosure see (viii).

(ix) Care should be taken when selecting and using an ammonia gas detection device and interpreting the measurement. Some devices directly measure an instantaneous concentration of ammonia, whilst others provide a time weighted average concentration measurement (eg accumulated measurement of ammonia ppm/time hrs = average concentration of ammonia). There is ongoing discussion as to which measurement provides the most accurate reflection of irritant levels of ammonia. Refer to Section 4 for available air quality monitoring devices.

Species Specific Recommendations:

Rabbits:

(i) As rabbits shed considerable amounts of hair, the extract ducts should be cleaned regularly to ensure continued efficiency of ventilation.

(ii) As rabbits are particularly sensitive to ammonia build-up, instantaneous ammonia concentrations inside rabbit enclosures (at the level of the rabbit nose) should be 10ppm or less.

3.3.4 Noise and Vibration.

The control of noise and vibration is important in the care of laboratory animals. Loud, intermittent and unfamiliar sounds are probably more disruptive than constant sounds. Prolonged noise over 100dB, or 160dB short-term, cause inner ear damage, noise-induced seizures and other problems to rodents.

Different laboratory animal species hear different pitch and loudness. Laboratory animals are sensitive to ultrasound, which can cause behavioural disturbances, and they are also able to hear frequencies that are inaudible to humans. Rodents communicate at 10-70kHz, compared with the human audible range of up to 20kHz. In addition, it is known that rats are particularly sensitive to ultrasound and that rodent neonates use ultrasound to communicate.

General Recommendations:

(i) Intense noise should be avoided as it can cause alterations to inner ear, gastrointestinal, immunological, reproductive, nervous and cardiovascular systems, as well as metabolic and behavioural aberrations.

(ii) Background noise (including ultrasound) should be kept below about 50dB (eg radio) and should be free of distinct tonal content.

(iii) Short exposure noise should be kept to less than 85dB.

(iv) Excessive noise and vibration most commonly arise from imperfectly balanced rotating or reciprocating machinery, particularly on start up. Machines that switch on and off intermittently may require special precautions as they may transmit vibrations over considerable distances. Vibrational stability is of greater concern for animal facilities located on the upper levels of a building. The density of ventilated racks will affect the noise level.

(v) Noise and vibration should be controlled in an animal facility through design and construction of the facility, and through the appropriate selection of equipment, and shielding and dampening devices.

Species Specific Recommendations:

Guinea Pigs and Rabbits:

(i) As guinea pigs and rabbits are easily startled by sudden noise, and may injure themselves in panic, care should be taken to minimise the generation of extraneous noise in the vicinity of these animal, therefore some form of low-level background noise in the animal room may be suggested.

3.3.5 Light

Mice, rats, rabbits and guinea pigs are either crepuscular or nocturnal. Their eyes are therefore adapted to dim light conditions. Light-induced retinal damage occurs principally in albino animals, even under normal lighting conditions (over 60lux), and may lead to blindness with exposure to light above 100lux for longer than 16 hours daily. Light intensity can influence aggressiveness and the incidence of cannibalism in rodents.

There is uncertainty as to whether laboratory rabbits are diurnal, nocturnal or crepuscular. It appears that external noise and scheduled feeding during the day can turn laboratory rabbits (and other animals) into predominantly diurnal animals.

The important aspects of light to consider are intensity, wavelength and photoperiod.

Minimum Standards for Lighting:

(i) The maximum allowable light intensity in an animal room is equivalent to 350lux at one metre height.

(ii) Animals must have the opportunity to withdraw to lower light intensities, especially those in top racks and albino animals.

(iii) Periods of light and dark must be provided to the animals each day.

General Recommendations:

(i) Light intensity in the animal room for safe and effective performance of routine animal care and laboratory activities should be considered.

(ii) Light intensity may vary considerably between top and bottom cages in a rack. Shelters or bedding should be provided within animal enclosures to enable animals to regulate their exposure to light. Light covers should be used where needed to diffuse and soften room lights.

(ii) Lighting should be provided by natural light or fluorescent light that duplicates the characteristics of sunlight.

(iii) Varied light / dark cycles are required to regulate breeding and circadian rhythms. Consideration should be given to providing a warning light outside animal rooms to indicate dark cycles because light interruptions during the dark phase may significantly skew endogenous rhythms. Animals may take 10 to 14 days to adapt to any change in photoperiod.

(iv) A red light source can be used for human activities during dark cycles. Alternatively, a separate room should be used for care-related activities.

(v) Whenever possible, lighting should be monitored at the room level and centrally controlled.

3.3.6 Emergency Plans and Alarms Systems

Minimum Standards for Emergency Plans and Alarms System:

(i) Animal facilities must be able to detect fire or the breakdown of essential climate control equipment such as ventilation and temperature control systems.

(ii) Animal facilities must have in place emergency plans.

General Recommendations:

(i) The heating, cooling and ventilation should be monitored to ensure that acceptable environmental conditions are maintained at all times. This is particularly important for individually ventilated cage systems, as fatal levels of carbon dioxide may accumulate within a couple of hours of ventilation system failure.

(ii) The operation of the alarm system should cause minimal disturbance to the animals where possible. For example, a tone generator can be used to reduce the frequency and decibel level of an existing alarm, or there is the 'silent' alarm, which is inaudible to small rodents. Most rodents cannot hear frequencies up to 1000kHz, whilst guinea pigs can hear frequencies from 200 to 2000kHz. Refer to Section 4 for available 'silent' alarm products.

3.4 Behaviour and Environmental Enrichment

Typical behavioural repertoires of these species include foraging, burrowing, climbing, nesting and nest building, social grooming and play, habitat manipulation and exploration, rest and communication. Addressing these behavioural needs is complex but involves the appropriate use of a combination of the following broadly classified conditions; (1) physical enrichment (items added to the environment), (2) social enrichment, and (3) structural enrichment (ie. modifications to the enclosure design).

3.4.1 Behaviour

Minimum Standards for Behavioural Requirements:

(i) Animals must be able to perform a variety of natural activities consistent with species specific behaviour, including the opportunity for sufficient exercise within their enclosure.

(ii) Breeding animals must be provided with the opportunity to nest.

(iii) Animal behaviour must be monitored closely to detect early signs of ill health, abnormal stereotypy or unexpected adverse effects during experimental protocols.

General Recommendations:

(i) Experimental protocols involving the modification of animal behaviour should comply with *The Australian Code.*

(ii) Young animals involve themselves in more play and exercise which is important for their development. Adequate exercise is important for skeletal and muscular development and maintenance. Exercise should be enabled through the provision of adequately sized enclosures, group housing and sometimes play objects.

3.4.2 Environmental Enrichment and Complexity

The aim of enrichment is to provide variety and stimulation in an artificial environment to enable normal animal behaviour without compromising experimental outcomes.

Mice and Rats:
The provision of environmental enrichment for mice and rats should mimic natural habitat and behavioural requirements including in particular tunnelling, foraging, climbing, social groupings and nesting. This encourages more social, content and easy to handle animals, particularly for rats held singly for experimental requirements.

As mice and rats have relatively poor eyesight, they rely heavily on their sense of smell and create patterns of urine markings to compartmentalise their environment or for territorial purposes. Provision of appropriate bedding is essential for these species.

It should be noted that stereotypic wire-gnawing in mice has been shown to be a form of displaced behaviour reflecting a lack of appropriate shelter (not a lack of provisions for gnawing) and can be easily corrected with shelter provision.

Guinea Pigs:
Vocalisation appears to play an important part in guinea pig social and sexual behaviour. They instinctively spend less time in open spaces, to prevent advertising their presence, preferring to sit or lie against the solid walls of their enclosure. They are poor diggers, but enjoy burrowing for concealment and protection of young. Guinea pigs like to shuffle and run in the floor pens, and young often chase each other. Guinea pigs will chew and eat plastics and other materials, but this generally does not appear to cause harm.

Rabbits:
The welfare of laboratory rabbits can undoubtedly be enhanced by enriching their environment and providing sufficient space for exercise, social interaction and play. The provision of environmental enrichment is particularly important for singly housed or caged rabbits, and the provision for adequate exercise is fundamental to normal skeletal and muscular development and maintenance of all laboratory rabbits.

Minimum Standards for Environmental Enrichment and Complexity:

(i) Ways of improving the environmental complexity to encourage and facilitate the natural behaviours of animals must be introduced, particularly for singly housed animals.

(ii) Enrichment items must not only meet the animals' needs, but must also be practical and safe for the animals.

General Recommendations:

(i) Care must be taken to monitor the worth of the enrichment items. Increased environmental enrichment should not be viewed as a substitute for clinical observation and close monitoring of the welfare of laboratory animals.

(ii) Prejudgements and preconceptions about how animals will interact with enrichment devices or materials should be avoided. The introduction of novel devices should be trialed as appropriate.

(iii) In general, the environmental complexity of housing can be increased by providing:

Physical Enrichment, for example:

- opportunity for the animals to retreat from light and modify their environment by the use bedding and nesting materials,
- a varied diet or alternative means of accessing food, such as by foraging, provisions for gnawing; and
- materials for specific behaviours (retreat, withdrawal, play) and exercise (eg piping or tubes, tins to hide in, exercise wheels, items/play objects to move about).

Social Enrichment for example:

- social companionship both with other animals (visual, audio and olfactory stimuli) and through human interactions. The transfer of some soiled nesting material into clean cages can reduce aggression following cleaning;

Structural Enrichment for example:

- transparent cages/boxes, provision of shelves, sufficient height of enclosures for rats, provision of exercise pens or increasing floor area for rabbits.

(iv) Animals should be observed for social interaction and signs of stereotypic gnawing, noise-induced circling (mice), barbering, fighting or excessive repetitive behaviours which may be abnormal stereotypies or stress-related behaviours.

Species Specific Recommendations:

Mice and Rats:

(i) Specific examples of methods of providing environmental enrichment are:

- Food treats and foraging activities: eg hard shelled nuts, pumpkin seeds for rats, sunflower and sesame seeds for mice; and
- Chewing, gnawing and shelter provisions eg. non-toxic gnawing blocks or wooden balls, golf balls cardboard rolls/tubes/boxes, old, clean plastic water bottles.

(ii) Consideration should be given to priority implementation of environmental and exercise devices for aggressive groups of males or singly housed mice and rats (eg exercise wheels for mice, PVC piping for rats). High top cages are mandatory for rats (see appendix 1) and are particularly important for singly housed rats to ensure that they are able to see out to gain social interaction.

Guinea Pigs:

(i) Specific examples of methods of providing environmental enrichment for guinea pigs are:

- Food treats and foraging activities - a daily supplement of hay is considered as a basic form of enrichment and fibre provision; and
- Hiding and chewing - upturned cardboard or plastic boxes, plastic tubes, sterilised softwood sticks.

Rabbits:

(i) Rabbits housed in cages should be provided with environmental enrichment.

(ii) Specific examples of methods of providing environmental enrichment for rabbits are:

- Varied food and other supplements for foraging, chewing, gnawing (and hiding and nest building) can be supplied to rabbits in cages or pens, eg daily provision of hay/straw, hay blocks, chew sticks, branches with leaves, small cardboard boxes, vegetables etc (see also Section 2.1); and
- Ledges or compartments on which to sit or to retreat. Ideally ledges should be 20-30cm above the cage or pen floor, and accommodated by the necessary cage height. Ledges and nesting boxes (outside the cage) will also increase the space available to the rabbits.

(iii) Enrichment in floor pen systems can be readily achieved with the use of hay bales, PVC pipes, boxes and compartments for elevation and concealment.

(iv) Rabbits undergoing experimental procedures in confinement or isolation should be given periodic access to an exercise area wherever possible.

3.5 Maintenance and Hygiene

Regular cleaning and maintenance and a high level of hygiene are essential for good husbandry and welfare. There is, however, a real danger of over cleaning cages used by pregnant and lactating animals. Such disturbances can result in cannibalism or mismothering. Odour marking is also an important activity in these species, and cleaning disturbances can cause a degree of social disruption.

Minimum Standards for Maintenance and Hygiene:

(i) Animal rooms and houses must be kept clean, tidy, vermin-proof and in good repair to facilitate effective cleaning.

(ii) Bedding in animal enclosures must be changed as often as necessary to keep animals clean, dry and comfortable.

General Recommendations:

(i) Decisions on frequency of cleaning should be based on cage system, type of animal, stocking densities, and the ability of ventilation systems to maintain suitable air quality. Alternation of partial cleaning (eg removal and replacement of soiled bedding) with full cleaning, permits some odour cues to remain in the cage and reduces the disturbance to the animals.

(ii) Care should be taken with the use of bleach and associated fumes, which may lead to disturbances of the young and mismothering.

(iii) Routines should be established for cleaning, washing, decontaminating or sterilising cages and accessories. The choice of detergents, disinfectants, washing and sterilisation systems should be based on the avoidance of contamination and denaturation of the material of the enclosure. Manufacturers' recommendations should be heeded. SOPs / Checklists should be established within an institution for routine maintenance and cleaning.

(iii) Any smell of ammonia should be investigated (See Section 3.3.3).

(iv) The movement of personnel between rooms of the animal facility should take into account 'clean' and 'dirty' areas, including the maintenance of differential air pressures where relevant.

(v) Climate control systems such as those that regulate ventilation and temperature should undergo regular maintenance. In addition, ventilation filters should be changed as required, and autoclaves and cage washers should be serviced regularly.

3.6 Handling and Basic Procedures

The behaviour of an animal during handling and the performance of experimental procedures depends to a considerable extent on the confidence and competence of its handler, which are developed through the application of good technique. Good technique should be unhurried, sympathetic and gentle but firm and safe for the animal and operator.

Minimum Standards for Handling and Basic Procedures:

(i) Institutions must ensure that animal house personnel and investigators have the training they require to handle animals competently and to carry out basic procedures as well as those approved as a result of applications to the AEC. This may involve facilitating access to relevant courses, supervision during training and promoting the awareness of SOPs.

(ii) Consideration must be given to the age and physiological state of the animal (eg pregnant, sick, immunocompromised) when carrying out procedures or handling animals.

Species Specific Recommendations:

Mice:

(i) Care should taken to avoid handling the last third of the tail of mice and lengthy periods of handling by the tail in general.

Guinea Pigs:

(i) Guinea pigs may appear nervous but rarely bite and can be easily handled. They should be forewarned before being approached to avoid stampeding. Care should taken to support the back and hindquarters when handling pregnant sows.

Rabbits:

(i) Care should taken to support the spine and hindquarters of rabbits, especially pregnant does.

(ii) A quiet approach and a darkened retreat area for rabbits (eg PVC pipe or box that can be suspended) is recommended when catching rabbits, particularly from pens.

(iii) Rabbits undergoing experimental procedures should be conditioned to human handling to reduce stress during procedures.

(iv) The training and rewarding of rabbits using positive reinforcement or 'treats' should be considered when performing procedures on rabbits.

3.7 Health Monitoring

It is important to the welfare of the animal colony to detect ill health and prevent the spread or establishment of infectious diseases. The level of monitoring will vary depending on the animals, the facility and the nature of the research conducted in the facility. Regular verification of the microbiological status of certain colonies for validation of research work and animal health and welfare may also be required.

Health monitoring encompasses microbiological, parasitological, serological and molecular diagnostics, analysis of breeding records and mortality rates, protocols for bringing animals and biological products into the facility, and phenotype reports. These details should be included in the health policy. The health policy should also include what action to take if an animal is suspected of ill health or abnormal behaviour.

Disease prevention is a combination of good management, knowledgable and cooperative research staff and an understanding of the epidemiology and biology of the pathological agents involved.

Examples of clinical monitoring sheets can be found in the various appendices. Records for animals in breeding facilities are discussed in Section 3.10 (iii).

Mice and Rats:

Specialist advice for health monitoring of mice and rat colonies should be sought from veterinarians and/or pathology laboratories. The use of sentinel animals should be considered and interpreted within the context of the size of the group and housing

system. Furthermore, not all conditions seen in laboratory mice and rats are of an infectious nature. Consideration should be given to problems with diet, husbandry and environmental control in addition to monitoring of infectious agents.

Rabbits:
Rabbits in cages may be difficult to assess for normal behaviours (as there may be inadequate space to carry out these behaviours) and changes in food and water intake may be the only early indicators of illness. Rabbits in pens should also be observed for changes in social interaction, in particular concerning subordinate animals. Evidence indicates that infectious disease spread in rabbits housed in groups in pens is no more of a problem than for animals housed singly in cages, providing high standards of care and monitoring are maintained. Certain diseases (eg *Pasteurella multocida* or 'snuffles') may in fact be reduced in penned rabbits, due to improved ventilation in pens in contrast to solid-walled cages. In addition, the occurrence of certain conditions with an underlying non-infectious cause such as hairballs, sore hocks and reversible bone thinning are rare or non-existent in penned rabbits when compared with their caged counterparts.

Minimum Standards for Health Monitoring:

(i) Health monitoring programs must consider the source and species of the animal, husbandry practices, the nature of the research being carried out in the facility, the movement of personnel and the risk to the colony.

(ii) Persons in charge of the facility must ensure that a health policy is developed and adopted to ensure that all animals are kept in optimal health and treatment is available for those animals not displaying normal behaviour.

(iii) Where an experiment is likely to have a negative impact on the health of the animals, details of animal monitoring and personnel responsibilities must be described in the project application submitted to the AEC.

(iv) The appropriate level of biohazard containment must be used for animals exposed to known infectious agents.

General Recommendations:

(i) This section is not intended to dictate comprehensive health monitoring programs. A laboratory animal veterinarian should be consulted about a program of regular monitoring of the health status of animals within a facility. The frequency and intensity of health and microbiological monitoring programs should be determined after consideration of defined risk factors.

(ii) Barrier housing aims to prevent infectious agents entering the barrier. Facilities and rooms should be classified according to the different levels of microbiological control required.

(iii) Where possible, the health status of all animals should be ascertained before the animals are brought into the facility. Animals of unknown health status should be quarantined or isolated and tested before being admitted to the facility.

3.8 Transportation

Minimum Standards for Transportation:

(i) The International Air Transport Association and AQIS must be consulted in the first instance for regulations pertaining to the respective air travel or export of mice, rats, rabbits or guinea pigs.

(ii) Food and water must be provided to the animals wherever possible during transport.

(iii) Animals must not be transported for more than 24 hours without food and water.

(iv) The sender must ensure that the animals to be transported are in good health.

(v) An assessment of the health and welfare of the animals must be made upon arrival.

(vi) Containers for domestic, local and internal transportation of animals must be:

Adequately ventilated (with reduced stocking rates in containers with filters);
Vermin- and escape-proof;
Durable (including crush-proof);
Sufficiently spacious (higher stocking densities than normal housing may be required to prevent injury);
Provided with appropriate bedding (for thermoregulation and impact absorption);
Clearly labelled.

General Recommendations:

(i) The general principles for animals obtained from interstate or overseas, transport of animals and admission of new animals into holding areas are covered in The Australian Code, which should be the first point of reference.

(ii) Guidelines for space, height, stocking density and ventilation requirements for non-air transport can be found in *'Housing for Laboratory Rats, Mice, Guinea Pigs and Rabbits'*; A.L. Hargreaves ANZCCART 2000.

(iii) Moist food (eg fruit, vegetables, mash or palatable glucose product) should be provided to avoid dehydration.

(iv) Rodents should be transported in compatible groups of familiar animals, formed at least 24 hours before transport commences.

(v) Sick or injured animals should be transported only for purposes of treatment, diagnosis or euthanasia.

(vi) Consideration should be given to the transport of pregnant animals.

(vii) Appropriate facilities for acclimatisation, isolation or quarantine should be provided. Newly-arrived animals may take up to two weeks to acclimatise, depending on the duration and mode of travel, which should be considered in the planning stage of scientific procedures. These animals should be closely monitored, especially guinea pigs as they may be reluctant to eat or drink from unfamiliar systems. Consultation with the animal technician or laboratory veterinarian may be required.

Species Specific Recommendations:

Guinea Pigs:

(i) Guinea pigs are prone to stress during transport and can stampede. This risk can be minimised by ensuring only 2-3 animals per container and provision of hay for hiding and cushioning.

Rabbits:

(i) Rabbits should be transported in insect-proof enclosures to prevent entry by vectors infected with myxomatosis or calicivirus.

3.9 Euthanasia

This code supplements *The Australian Code* and provides further guidance and recommendations on the choice of method of euthanasia for the species and age of the animal. The 2001 ANZCCART publication; *Euthanasia of Animals for Scientific Purposes* has been the main source of this information in an effort to ensure that the most suitable and humane methods are adopted in the pursuit of 'best practice'. This reference should be consulted for further guidance on the choice and details of acceptable methods of euthanasia.

Minimum Standards for Euthanasia:

(i) The general principles of the current edition of *The Australian Code* must be adopted in the first instance;

- *'When it is necessary to kill an animal, humane procedures must be used. These procedures must avoid pain or distress, be reliable and produce rapid loss of consciousness until death occurs. The procedures should also be compatible with the scientific or educational aims.*
- *The procedures must be performed only by competent persons approved as competent by AEC or under direct supervision of a competent person.*
- *Animals should be killed in a quiet, clean environment, away from other animals where possible. Death must be established before disposal of the carcass.*
- *Dependent neonates of animals being killed must also be killed or appropriate 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 assured.'*

(ii) The methods listed as 'not acceptable' in Appendix 2 of this code are regarded as either inhumane or associated with practical problems. These methods are not permitted in Victoria.

General Recommendations:

(i) The method of euthanasia, whether chemical or physical, must satisfy objective criteria to ensure the procedure is humane and practical, as set out in the *AVA policy on euthanasia*, namely:

- *'Death without signs of panic, pain or distress;*
- *Minimum time to loss of consciousness;*
- *Reliability and reproducibility;*
- *Simple, relatively maintenance-free mechanical equipment;*
- *Minimal emotional effects on the observer and operator; and*
- *Safety for operators and observers.'*

The various potentially acceptable methods of euthanasia are tabled in Appendix 2. A method described as 'acceptable but with reservations' is one that fails to meet all the AVA criteria. These methods should be avoided unless the AEC is satisfied regarding

scientific justification and the competence of specific personnel to carry them out. An 'acceptable' method satisfies all the AVA criteria and is one of the preferred methods for the particular species.

(ii) The species differ in their capacity to experience pain during the various stages of development. For example, guinea pigs are more developed and sentient at birth than rats. Recommended methods of euthanasia for fetal or neonatal animals are covered in the guidelines in Appendix 3.

3.10 Monitoring and Records

Whether for the purpose of inspection by the AEC or government authority, for OGTR or AQIS requirements, for breeding, experimental or trouble-shooting purposes, it is important to maintain complete and thorough records on all animals. In addition, SOPs may be useful to ensure that monitoring in animal care facilities is consistent and comprehensive. This section in particular should be read in conjunction with the various references to 'monitoring' in the current *Australian Code*.

Examples of recommended monitoring sheets can be found in Appendices 4-10. These can be modified to meet the specific needs of the animals, animal house personnel or investigators, but should demonstrate important considerations of animal health and welfare.

For the purpose of this Code, monitoring means assessment and recording of events, data or effects as per the minimum standards. Unless specified in the AEC approved project application, the primary responsibility for monitoring the health and welfare of animals during an approved project lies with the assigned chief investigator of the project.

Minimum Standards for Monitoring and Records:

(i) A system of assessment and recording of animal breeding or use must be implemented.

(ii) For the purpose of inspection, animal records must be retained for a minimum of four years.

(iii) Relevant records (including computer records) must be readily accessible for inspection.

(iv) Any transfer or sharing of the responsibility for monitoring animal health and welfare between animal house personnel and research investigators, especially following invasive procedures, must be recorded.

Recommendations:

(i) Monitoring should include the assessment and recording of:

- Animals issued to projects and AEC approval dates;
- Newly-arrived animals/animals received for health and acclimatisation;
- Room temperature and humidity (ideally constantly);
- Animals that are identified as sick or recovering post-procedure;
- Pregnant and lactating animals and litters;
- Breeding parameters (see 3.10 iii);
- Expected or unexpected adverse effects on all animals, including genetically modified or cloned animals (see 3.10 v);
- Deaths and culls; and
- Training of animal technicians and investigators in handling, basic procedures, obligations under the Codes, The Act, and institution policy.

(ii) Numbers of animals displaying adverse effects, pain, distress or ill health must be monitored daily, and ideally should be recorded on monthly or annual sheets to enable trends to be identified and possible disease or adverse phenotypes to be detected and reported if necessary.

Refer also to Appendix 10 for an example phenotype report.

(iii) Breeding facilities must maintain adequate records to allow the effective management of the colonies, including detection of the origin and spread of disease. Records consistent with The Australian Code should include:

- *'The source, care, allocation, movement between locations, use and fate of all animals;*
- *Details of any disease;*
- *The fertility, fecundity, morbidity and mortality in breeding colonies; and*
- *The health status, genetic constitution and the physical environment of the animals'.*

Refer also to Appendices 7, 8 and 9.

(iv) To centralise and communicate effective monitoring and records, computer databases should be used.

(v) Records of unexpected adverse effects associated with phenotype or procedure should be maintained and, preferably, disseminated within the institution. Refer also to Appendix 10 for an example phenotype report.

4. Further Information Sources

Please refer to the comprehensive 'information sources' section in the current edition of *The Australian Code*. In addition, reference publications, guidelines or codes, consulted or mentioned during the development of this code are provided below.

- *A review of enrichment techniques for laboratory rodents. D.Figa, University of Sydney. 2004*
- *Code of practice for the housing and care of animals used in scientific procedures parts 1 and 2.* www.homeoffice.gov.uk/docs/hcadb5.html
- *Code of Practice for the Housing of Animals in Designated Breeding and Supplying Establishments.* www.homeoffice.gov.uk/docs/hcadb6.html
- *Code of Practice for the Humane Killing of Animals* under Schedule 1 to the Animals (Scientific Procedures) Act 1986 www.homeoffice.gov.uk/docs/hc193.html
- *Comfortable Quarters for Laboratory Animals*. V and A Reinhardt. 9th edition, 2002. Animal Welfare Institute www.awionline.org/pubs/cqindex.html
- *Environmental enrichment information: resources for laboratory animals.* www.nal.usda.gov/awic/pubs/enrich/intro.htm
- *Euthanasia of Animals Used for Scientific Purposes*. Published by ANZCCART 2001. www.adelaide.edu.au/workp.html
- *Guide for the Care and Use of Laboratory Animals*. National Research Council (USA). National Academy Press.
- *Guide to the Care and Use of Experimental Animals*. Volume 1 and 2. Canadian Council on Animal Care. www.ccac.ca
- *Guidelines for the Housing of Rabbits in Scientific Institutions*. NSW Animal Research Review Panel http://www.agric.nsw.gov.au/reader/animal-care
- *Guidelines for Transgenic Research*. Canadian Council on Animal Care. www.ccac.ca
- *Housing for Laboratory Rats, Mice, Guinea pigs and Rabbits*. A.L Hargreaves. ANZCCART 2000
- *Individually ventilated cages and rodent welfare*: Report of the 2002 RSPCA/UFAW rodent welfare group meeting. Email: Research_Animals@rspca.org.uk for a copy.
- *International Air Transport Association (IATA) Live Animal Regulations*: worldwide standards for transporting animals by commercial airlines. www.iata.org/cargooperations/liveanimals/index
- *Macro vs Micro environment; Static vs Ventilated Cages; Facility Design vs Practices*. Dr Beth Ford. ANZLAS Conference 2003.
- *National Institutes of Health (NIH) Design Policy and Guidelines, VOLUME 3 Animal Research Facilities*. NIH (USA). www.nih.gov/research/animals
- *Office of Gene Technology - guidelines for the certification of PC2 animal facilities.* www.ogtr.gov.au
- *Recommendations for Euthanasia of Experimental Animals Part 1 and 2.* Report of a United Kingdom Working Party. Laboratory Animals, 30:293-316, 1996; 31: 1-32, 1997.

- *Refinement of housing conditions and environmental enrichment for laboratory animals.* www.awionline.org/lab_animals/biblio/laball.htm
- *Refinement and Reduction in Production of Genetically Modified Mice.* Report of the BVAWF/FRAME/RSPCA/UFAW Joint working group on refinement. Laboratory Animals n37, Supplement 1, 2003.
- *Report of the AVMA Panel on Euthanasia.* Published by the American Veterinary Medical Association (AVMA) www.avma.org/respources/euthanasia.pdf
- *The Biology and Medicine of Rabbits and Rodents*, 3rd edition. Harkness J.E. and Wagner J.E. (1989) Lea & Febiger, Philadelphia PA USA.
- *The Mouse and The Rat*, 1993 ANZCCART fact sheets. www.adelaide.edu.au/workp.html
- *UFAW handbook on the care and Management of Laboratory Animals. 7th Edition, Vols 1 and 2*, Poole,T.B (ed) (1999) Universities Federation for Animal Welfare. Blackwells Scientific and Technical, Harlow UK.

Ammonia and other gas monitoring devices:

- www.afcintl.com/gasdect.htm

“Silent” alarms:

- ‘Silentone’ and ‘Klaxon’ products: COMPMED archives/ ‘Laboratory Animals’ Buyers Guide.

APPENDIX 1. MINIMUM STANDARDS FOR HOUSING OF LABORATORY MICE, RATS, GUINEA PIGS AND RABBITS.

Recommendations are in brackets and italics

Figures in this appendix are based on various international guidelines and codes, and current acceptable minimal standards of practice in Victoria.

Table 1. Minimum Standards

Species	Single or group housing or breeding animals	Animal weight (g)	Min floor area * (cm^2 per animal)	Min height ** (cm)	Room temp (°C)	Relative humidity (%)	Room vent (ACH)	Max light *** (lux)
MICE	single	Any	200	**	18-24	40-70	*(10-20)*	350
	group	<30	60	**				
		31-40	70					
		>40	100					
	breeding	Pair	300	**				
		Extra females + litter	150 per extra female					
RATS	single	<250	500	**	18-24	40-70	*(10-20)*	350
		250-550	700					
		>550	800					
	group	<150	150	**				
		150-350	225					
		351-550	300					
		>550	450					
	breeding	F + litter	800	**				

GUINEA PIGS	**single**	<250	700	20	18-24	40-70	*(10-20)*	350
		250-550	900	23				
		>550	1000	23				
	group	<250	300	20				
		250- 550	450	23				
		>550	600	23				
	breeding	F + litter	1200	23				
		per F in harems	1000	23				
RABBITS	**See over page**				<30 *(15-24)*	40-70	*(15-20)*	350

* Minimum area includes the area of any shelving/devices in the animal enclosure that doesn't reduce the total available area.

** Minimum height for mice and rat enclosures must allow mice and rats to stand upright on hind legs and ideally look out from the enclosure, especially if singly housed. This may be achieved by using either suitably high opaque or transparent cage enclosures with flat lids or high top lids.

***Maximum light intensity recommended for albino animals is100 lux for 16 hours continuously.

Table 2. RABBITS: Housing Space Minimum Standards:

Cages or Pens:

Single or group housing or breeding animals	Animal weight* (kg)	Min. floor area ** (cm^2 per animal)	Min height *** (cm)
Single	<2	2000	***
	2-4	4000	
	4-6	5400	
	>6	6000	
Group	<2	1300	***
	2-4	2600	
	4-6	3300	
	>6	4000	
Female + litter	Any weight	9300 per female + litter	***

* The animal weight provides an indication of the age and activity of the rabbits which is the important determinant of space requirements.

** Minimum area includes the area of any shelving in the animal enclosure that doesn't reduce the total available area. Enclosures must be wide and long enough to permit rabbits to lie fully outstretched ie. minimum of 80cm clear space in at least one direction, and to provide a clear area to facilitate 1-3 complete hops for normal exercise requirements.

*** The minimum height must refer to a cage/pen high enough for rabbits to stand upright unhindered (ie with ears not touching the roof).

APPENDIX 2: METHODS OF EUTHANASIA FOR POST-NEONATAL LABORATORY MICE, RATS, GUINEA PIGS AND RABBITS. Based on *'Euthanasia of Animals Used for Scientific Purposes'*; ANZCCART 2001.

Table 1. RATS AND MICE

Technique	Acceptable	Acceptable with reservations	Not acceptable
Chemical: inhalant	▪ Carbon dioxide [1]	▪ Halothane, Isoflurane, Methoxyflurane [2]	▪ Ether [2, 3] ▪ Hydrogen cyanide [2, 4] ▪ Carbon monoxide [2] ▪ Nitrogen [4] ▪ Chloroform [2]
Chemical: injectable	▪ Pentobarbitone sodium i/p (i/c after sedation) Xylazine/Ketamine ▪		
Physical	▪ Cervical dislocation [5] if less than 150 g	▪ Decapitation [1, 4, 5,6] ▪ Stunning and exsanguination [4, 5,6]	▪ Microwave irradiation – not yet proven to be humane [1] ▪ Decompression [1, 4] ▪ Asphyxia [1, 3, 4] ▪ Rapid freezing[1, 3]

[1] Requires specialised equipment
[2] Occupational health and safety issues
[3] Inhumane
[4] Aesthetically unpleasant
[5] Requires specialised training +/- sedation.
[6] Only for justified tissue collection.

i/c (intracardiac), i/p (intraperitoneal), i/v (intravenous)

Table 2. GUINEA PIGS

Technique	Acceptable	Acceptable with reservations	Not acceptable
Chemical: inhalant	▪ Carbon dioxide [1] (for animals <600g)	▪ Halothane, Isoflurane, Methoxyflurane [2] ▪ Nitrous oxide (must be used with other inhalants) [2]	▪ Ether [2, 3] ▪ Hydrogen cyanide [2, 4] ▪ Carbon monoxide [2] ▪ Chloroform [2]
Chemical: injectable	▪ Pentobarbitone sodium i/p (or i/c after sedation)	▪ Intravenous agents are acceptable only with i/v catheter placement.	
Physical	▪ Cervical dislocation [5] if less than 150 g	▪ Stunning and exsanguination [4, 5,6]	

[1] Requires specialised equipment
[2] Occupational health and safety issues
[3] Inhumane
[4] Aesthetically unpleasant
[5] Requires specialised training +/- sedation.
[6] Only for justified tissue collection.

i/c (intracardiac), i/p (intraperitoneal), i/v (intravenous)

Table 3. RABBITS

Technique	Acceptable	Acceptable with reservations	Not acceptable
Chemical: inhalant	None recommended	▪ Halothane, Isoflurane, Methoxyflurane [2] ▪ Nitrous oxide (must be used with other inhalants) [2]	▪ Chloroform [2, 3, 4] ▪ Carbon dioxide [1, 3, 4] ▪ Hydrogen cyanide gas [2, 4] ▪ Carbon monoxide [1, 2]
Chemical: injectable	▪ Pentobarbitone sodium i/v or i/p	▪ Ketamine with a premedicant such as acetylpromazine or xylazine	▪ Ketamine alone [3] ▪ Magnesium sulphate, Potassium chloride [3]
Physical	None recommended	▪ Stunning and dislocation [4, 5,6] ▪ Captive bolt [1, 4, 5] ▪ Neck dislocation [5] (should only be used if anaesthetised first) ▪ Decapitation [1, 5] (should only be used if anaesthetised first)	▪ Neck dislocation without anaesthesia [3, 4] Decapitation without anaesthesia [1, 3, 4] ▪

[1] Requires specialised equipment
[2] Occupational health and safety issues
[3] Inhumane
[4] Aesthetically unpleasant
[5] Requires specialised training +/- sedation.
[6] Only for justified tissue collection.

i/p (intraperitoneal), i/v (intravenous)

APPENDIX 3 GUIDELINES FOR THE EUTHANASIA OF FETAL AND NEONATAL MICE, RATS, GUINEA PIGS AND RABBITS

The following guidelines are suggested to assist in reviewing proposals which involve the use of rodent and rabbit fetuses or neonates. This information can be used as guidelines for investigators, animal house staff and Animal Ethics Committees. In all cases, the person performing the euthanasia must be fully trained in the appropriate procedures.

Neural development up to 60% gestation is considered minimal and pain perception is considered unlikely. Euthanasia of the mother or removal of the fetus should ensure rapid death of the fetus due to loss of blood supply and non-viability of fetuses at this stage of development.1 Whilst hypothermia is known to act as an anaesthetic2 to a certain extent, it is still a controversial method of euthanasia, as is rapid freezing (eg. immersion in liquid nitrogen). Monitored hypothermia, followed by decapitation or rapid freezing (eg. immersion into liquid nitrogen) to ensure euthanasia of fetuses or neonates without fur and less than 4 grams is considered acceptable by some references3. Euthanasia by CO2 asphyxia is slow and unacceptable for neonates.

Mice and Rat Fetuses

(i) Fetuses 15 days in gestation to birth: the literature on the development of pain pathways suggests the possibility of pain perception at this time. Whereas fetuses at this age are resistant to inhalant anaesthetics including CO2, euthanasia may be induced by the skilful injection of chemical anaesthetics. Although aesthetically unpleasant, decapitation with surgical scissors or scapel, or cervical dislocation are humane and acceptable physical methods of euthanasia. When chemical fixation of the whole fetus is required, fetuses (with fur or greater than 4g) should be anaesthetised prior to immersion in or perfusion with fixative solutions. Anaesthesia may be induced by monitored hypothermia2 of the fetus, by injection of the fetus with a anaesthetic agent, or by deep anaesthesia of the mother with a chemical agent that crosses the placenta, e.g. pentobarbital. The institute veterinarian should be consulted for considerations of fetal sensitivity to specific anaesthetic agents. When fetuses are not required for study, the method chosen for euthanasia of a pregnant mother must ensure rapid death of the fetus.

Mice and Rat Neonates

(i) Up to 10 days of age: Acceptable methods for euthanasia of neonatal mice and rats include: injection of chemical anaesthetics (e.g. pentobarbital), decapitation, or cervical dislocation. Anaesthesia should precede immersion or perfusion with chemical fixatives (including liquid nitrogen) if the fetus is greater than 4g. Anaesthesia may be induced by injectable anaesthetics or hypothermia and the institute veterinarian should be consulted for appropriate agents and dosages.

(ii) Older than 10 days: Follow guidelines as per Appendix 2.

Guinea Pig and Rabbit Fetuses

(i) Embryos or fetuses: Literature on the development of pain pathways suggests that there may be pain perception consistent with development of the functional brain occurs from 60% gestation. Although aesthetically unpleasant, decapitation is an acceptable method for killing these forms. It is preferable to use a guillotine for decapitation.

Guinea Pig and Rabbit Neonates

(i) Guinea pig neonates are well developed, sentient animals from birth. Rabbit neonates, in this context, are newborn rabbits up to 10 days old. Intraperitoneal injection with an overdose of an appropriate anaesthetic agent is the preferred method for euthanasia of these neonates. Cervical dislocation is an acceptable method for guinea pigs and rabbits less than 100grams bodyweight.

(ii) Older than 10 days: Follow guidelines as per Appendix 2.

1 "When ovarian hysterectomies are performed, euthanasia of fetuses should be accomplished as soon as possible after removal from the dam. Neonatal animals are relatively resistant to hypoxia." 2000 Report of the AVMA Panel on Euthanasia, JAVMA 218:688.

2 Phifer CB, Terry LM. 1986. Use of hypothermia for general anaesthesia in preweanling rodent. Physiol & Behav 38:887-890.

3. "Recommendations for euthanasia of experimental animals. " Working Party Report 1996. Laboratory Animals, 30:293-316. (refer to www.lal.org.uk/workp.html)

APPENDIX 4 EXAMPLE ANIMAL MONITORING SHEET

AEC Project Number:	Investigator Name and Phone Contact (BH and AH):
Animal – ID Number:	Species/Strain
Animal details (sex/age etc)	Comments:

- Each animal is examined and observed for abnormalities at each time point (weekly or daily as appropriate)
- Observations are recorded in the table
- Normal clinical signs are recorded as "N"
- Abnormalities are recorded as "A" and severity is scored in brackets eg Breathing: A (3) (see over page)
- Comments concerning abnormalities are recorded in the comments section of the table
- Additional observations tailored to the monitoring requirements for each animal experiment are to be added at "Other"

CLINICAL OBSERVATION (N or A)	DATE						
UNDISTURBED							
Coat							
Activity							
Breathing							
Movement/gait/trembling							
Eating							
Drinking							
Alert/sleeping							

ON HANDLING							
Alert							
Body condition							
Bodyweight (g)							
Dehydration							
Eyes							
Faeces							
Nose							
Breathing							
Urine							
Vocalisation							
OTHER (specify)							
COMMENTS							
INITIALS:							

_____ _____

Signature of (Chief) Investigator Date

CLINICAL SIGNS SEVERITY SCORE

SIGNS	0	1	2	3
Activity	normal	isolated, abnormal posture	huddled/inactive OR overactive	moribund OR fitting
Alertness/ sleeping	normal	dull or depressed	little response to handling	unconscious
Body condition*	normal	thin	loss of body fat, failure to grow	loss of muscle mass
Body weight*	normal weight and growth rate	reduced growth rate	chronic weight loss >15% OR failure to grow	acute weight loss>10% chronic weight loss 20% OR failure to grow & weight loss
Breathing	normal	rapid, shallow	rapid, abdominal breathing	laboured, irregular, skin blue
Coat	normal	coat rough	Unkempt, wounds, hair thinning	bleeding or infected wounds, or severe hairloss or self mutilation
Dehydration	none	skin less elastic	skin tenting	skin tenting & eyes sunken
Drinking	normal	increased OR decreased intake over 24 hrs	increased OR decreased intake over 48 hours	constantly drinking OR not drinking over 24 hours
Eating	normal	increased OR decreased intake over 24 hours	increased OR decreased intake over 48 hours	obese OR inappetence over 48 hours
Eyes	normal	wetness or dullness	discharge	eyelids matted
Faeces	normal	faeces moist	loose, soiled perineum OR abnormally dry +/- mucus	running out on handling OR no faeces for 48 hrs OR frank blood on faeces
Movement/ gait	normal	slight incoordination OR abnormal gait	incoordinated OR walking on tiptoe OR reluctance to move	staggering OR limb dragging OR paralysis

Nose	normal	wetness	discharge	coagulated
Urine	normal		abnormal colour/volume	no urine 24 hrs OR incontinent, soiled perineum
Vocalisation	normal	squeaks when palpated	struggles and squeaks loudly when handled/ palpated	abnormal vocalisation
Other				

* these criteria may not apply in some situations (eg tumor growth, obesity/metabolic studies)

SPECIAL HUSBANDRY REQUIREMENTS**

EUTHANASIA/HUMANE EXPERIMENTAL ENDPOINT CRITERIA**

CLINICAL SIGN	ACTION

** as approved by the AEC, relevant to each specific situation

SCIENTIFIC MEASURES (ie data or tissues to be collected as part of the experimental use)
(eg animals that are killed should be weighed and have their bodies placed in labelled bags and refrigerated)

Reference: Morton, D.B. (1997) A scheme for the recognition and assessment of adverse effects in animals. In: Developments in animal and veterinary sciences, 27. *Animal Alternatives*, Welfare and Ethics. pp 235-240. Eds van Zutphen, L.F.M. and Balls, M. Elsevier Science B.V.

APPENDIX 5 EXAMPLE WELFARE ASSESSMENT SCORE AND JUDGEMENT SHEET

This is a general assessment for all animals, which may be altered for each individual animal or research project.

Animal ID:	**D.O.B:**							
AEC Project No:	**Date of Procedure:**		**-2**	**-1**	**0**	**1AM/PM**	**2**	**3**
Appearance/colour	Normal	0						
	Ruffed	1						
	Hunched/trembling	2						
	Listless	3						
Surface temperature and mucous membrane colour	Congested	2						
	Pale pink	2						
	Normal/warm	0						
Natural activity	Eating	0						
	Drinking	0						
	Observant	0						
	Active	0						
Reflexes/respond to touch/pain	Still	3						
	Normal withdrawl	0						
	Aggressive	3						
	Vocal	2						
Wound healing	Normal	0						
	Discharge	1						
	Discharge ++	2						
	Open Wound	3						

Extra info		0						
		1						
		2						
		3						
	SCORE							
Post procedure/ surgery requirements (tick if provided)	Pain Relief							
	Antibiotics							
	Fluids							
	Hand feeding							
	Mushy Food							

Judgement:

0-4 = Good
5-8 = Fair
9-12 = Poor

Comments:

Action (if any) required:

_____ _____

Signature of (Chief) Investigator Date

APPENDIX 6 EXAMPLE MONITORING SHEET FOR ANIMALS AFTER SURGERY OR INVASIVE PROCEDURES

This is a general assessment for all animals, which may be altered for each individual animal or research project.

AEC Project Number:	Investigator Name and Contact:
Animal - Number:	Start Date & Surgery/Procedure Date:
Procedure:	Comments:

OBSERVATIONS (Day in relation to surgery)	**-3**	**-2**	**-1**	**0**	**1 AM PM**	**2 AM PM**	**3**
DAY / DATE / TIME							
Eating							
Drinking							
Normal Walking							
Vocalisation							
Grooming							
Staggering							
Shivering/trembling/twitching							
Hunched-up Appearance							
Discharge from the surgical site							

These daily observations should start 3 days before surgery.
On the day of surgery/procedure and for 2 days after surgery/procedure, observations should be made each morning and afternoon.
From day 2 post-surgery/procedure observations should then revert to a daily basis.

Please Note:

1. Analgesia should be administered for the first 12 and/or 24 hours and thereafter as determined by daily observation, any evidence of pain and speed of return to normal behaviour.
2. If any abnormal behaviour is observed or if there is discharge from the wound, please contact your Animal Welfare Officer or your Animal House Manager.

APPENDIX 7 EXAMPLE DAILY AND MONTHLY BREEDING RECORD SHEET

Animal Facility Name:	Animal Facility Staff Name and Phone Contact:
Animal -Species:	Strain/Genetic description (indicate * if new GM strain)

Day	Cage no.	Live born	Still born	Neo-natal culls	Neo-natal deaths	Males weaned	Females weaned	Ave wt (g)	Wean date	Comments
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										
16										

17										
18										
19										
20										
21										
22										
23										
24										
25										
26										
27										
28										
29										
30										
31										
Total										

Signature of Animal Facility Staff _____ Date _____

APPENDIX 8 EXAMPLE MONTHLY STOCK INVENTORY FOR LICENCE CONDITIONS

Animal Facility Name:	Animal Facility Staff Name and Phone Contact:
Months of Year:	Comments:

Month	Species (M, R, GP, Ra) **	Source of Animals (P, AP, IP, OP)	X = No. Breeding and Stock Animals (existing lines***)	Y= No. Animals allocated to a AEC approved project	Z =No. animals NOT breeding nor allocated to a project and destroyed	Total = X + Y+ Z	No. live animals sold/ delivered to another licensed institution	Date of delivery	Destination

P: bred at this licensed premises
AP: bred at another licensed premises
IP: bred at an interstate premises
OP: bred overseas (note retain name and address of OS supplier)

** Specification of strain / genetic description (optional) but do indicate * if new GM strain
*** not final progeny that get allocated to a project

_____ _____

Signature of Animal Facility Staff Date

APPENDIX 9 NUMERICAL SCORE SHEET FOR ASSESSING NEONATAL GENETICALLY MODIFIED RODENTS.

AEC Project #:	**Animal ID/Strain*:**							
Investigator:	**Date:**							
Observer:	**Age (days):**							
Appearance/colour	Normal (pink)	0						
	Pink/blue abdomen	1						
	Pink/pale extremities	2						
	Blue/pale	3						
Surface temperature	Warm	0						
	Intermediate	1						
	Cold	2						
Natural activity	Wriggling ++	0						
	Wriggling +	1						
	+/-	2						
	Still	3						
Reflexes/respond to touch	+++ righting reflex	0						
	++ righting reflex	1						
	+	2						
	-	3						
Milk in stomach	++	0						
	+	1						
	-	2						

TOTAL SCORE (Neonate)	0-13							
Parameter (mother)	NB Include Milk Score							
Nest building	Good nest making	0						
	Some nest making	1						
	No nest	2						
Retrieval of young	Always	0						
	Sometimes	1						
	Never	2						
TOTAL SCORE (Mother)	0-6							

use * to identify new GM strain

Judgement: neonate
0-4 = Good
5-8 = Fair
9-12 = Poor
Always assess maternal factors as well. Maternal score 5-6 = Will these animals need fostering?

Comments:

Actions (if required):

(Form also available from: www.LAL.org.uk/pain/pain3.html)

APPENDIX 10 EXAMPLE PHENOTYPE REPORT FOR GENETICALLY MODIFIED ANIMALS

The main purpose of this report is to assist with the monitoring and assessment of the impact of genetic modification upon the health and welfare of the affected animals. Please provide information consistent with this purpose (ie detailed descriptions of in vitro methodology are not desired).

Please use **lay language** or provide glossary definitions.

Project Details

1. Dept AEC Project No.:			
2. OGTR/IBC Ref. No.			
3. Project Title:			
4. Start Date*:		**Finish Date*:**	
5. Chief Investigator:			
Department:			

* Relates to approved projects.

Animal Details

5. GM Animal			
Species:			
*** Strain/genetic description:** indicate * if new GM strain		Background Strain:	

Source: (ie in-house or specified external laboratory source)	
What is the health profile of the source colony? Provide the most recent serology report	

6. How much is known about the biological characteristics/phenotype of this strain?
Indicate by selecting one of the following:

☐ **Well characterised**

☐ **Partially-characterised/some information available**

☐ **Unknown**

DECLARATION BY CHAIRPERSON OF AEC

I certify that this report has been considered and accepted by the Animal Ethics Committee at the meeting on .....(date)

....

Chairperson's signature AEC Date

Glossary

Word	Lay explanation

7. **Genetic alteration:** (Briefly describe which gene has been added /deleted/ altered)	
Affected organs/tissues: (eg gene expressed in liver only)	
Is animal health, welfare, breeding or lifespan likely to be affected?	
What abnormalities are known to exist (or do you expect) in these animals?	

8. Clinical Observations

Comparison of modified/cloned animals with non-modified littermates is desirable.

- Supply a record of clinical observations made on a representative sample of the GM animal(s). Observations which are to be included can be found in an Animal Monitoring Sheet.
- Minimum period for observation record is 3 months; life-long data to be included where possible. If supplying "average" data, indicate number of animals observed and a measure of the variability of the data.

9. Phenotype

- Briefly detail any other observations which have been made to characterise the new strain (ie behaviour, physiology, reproductive or developmental measures)

10. Minimisation of Pain or Distress

- Describe any adverse affects, pain or distress, and/or unexpected mortality, the causes if known and how these problems were resolved. If none this should be indicated.

11. Special husbandry or animal care requirements specific for the new GM strain.

- If these are necessary, please provide details.

12. Humane euthanasia and experimental endpoint criteria.

- What objective criteria will be used to determine when an animal will be humanely killed or removed from an experimental study prematurely?

Please note, a "Request for Minor Amendment" must be submitted to your Animal Ethics Committee on the current form if:

- **you anticipate using greater numbers than requested; or**
- **there have there been any minor changes in procedure; or**
- **there have been any changes to personnel/staff changes including new honours/PhD students.**

If the modifications to procedures are considered significant you will be required to complete a new application.

==========

CERTIFICATION OF THE CHIEF INVESTIGATOR Signature signifies that the Chief Investigator understands the requirements of the Prevention of Cruelty to Animals Act 1986 & Regulations 1997 and the NHMRC Australian code of practice for the care and use of animals for scientific purposes governing the use of animals for research and teaching. Signature further certifies that the investigator will continue to conduct the project in full compliance with the aforementioned requirements.

_____ Date_____

Signature of Chief Investigator