



This document has been developed by The Australian National University's (ANU) Research Ethics Office. It has been endorsed by the ANU Animal Experimentation Ethics Committee (AEEC). It is designed to provide guidance regarding current best practice to institutional animal users and carers on the care and use of animals for scientific purposes. It has been prepared in consultation with the Australian code for the care and use of animals for scientific purposes 8th edition 2013.

Document 019: Laboratory Rodent Husbandry and Care Standards V1.0

Background

The following is an extract from the Australian Code of Practice for the care and Use of Animals for Scientific Purpose 8th Edition 2013:

2.5.2 Animal carers must, within the scope of their responsibilities:

- i. Apply the principles of the Code in all aspects of the care of animals (see Section 1)*
- ii. Follow relevant policies and procedures established by the institution and the AEC (See Clauses 2.1.5 [iv] and [v])*
- iii. Undertake activities in accordance with the conditions and requirements of approval from an AEC*
- iv. Take measures to ensure that the animals' environment and management are appropriate for the species and the individual animal, and support the animals' wellbeing*
- v. Ensure that steps are taken to safeguard animal wellbeing by avoiding and minimising harm, including pain and distress, to the animals*
- vi. Consider the application of Replacement, Reduction and Refinement (the 3Rs) in all aspects of the care of animals for which they are responsible*
- vii. Ensure that their duties are performed competently*
- viii. Liaise with investigators and relevant project team members on all matters relevant to the wellbeing of the animals involved*
- ix. Maintain records of the care of animals*
- x. Report to the AEC as required*

2.5.3 If more than one person is responsible for the care of animals (e.g. animal technicians caring for animals in one or more animal breeding and holding facility, team of animal technicians and researchers caring for animals in a project, team of researchers and wildlife carers involved in the care of wildlife in a research project, several teachers and students caring for animals in a school), a person must be identified who has ultimate responsibility for the care of those animals. Depending on the situation, this person may be the facility manager, or the investigator with ultimate responsibility for a project. Identification of a person with ultimate responsibility for the care of animals does not relieve the individual responsibility of each person who provides care for animals.



Definitions

Animal Carer	any person involved in the care of animals that are used for scientific purposes, including during their acquisition, transport, breeding, housing and husbandry.
AEC	Animal Ethics Committee – at the ANU known as the “AEEC” or Animal Experimentation Ethics Committee.
Visual Checks	The visual checking of animals is, for the purpose of this document, defined as examination of the animals in their cage environment at the rack level. This should include an assessment that their behaviour is normal given the time of day/situation in which they are assessed, cage condition, food/water provision and any signs of ill health. Care should be taken not to disturb vulnerable animals such as pregnant or lactating females with young.

General Information and Considerations

General Care

All animals must be visually checked daily for basic requirements including food and water availability, cage condition and a check for signs of illness or injury.

It is not a requirement that animals are disturbed from nesting/sleeping for daily cage checks unless there is a specific cause for concern (e.g. animals under experiment, an unwell animal, poor cage condition).

If an animal is found to be unexpectedly unwell it must be recorded and reported relevant to the severity of the condition and the status of the animal (i.e. under experiment, breeding or stock animal). Veterinary advice is available 24/7 and examination can be arranged for any animals that show illnesses of concern, where there is a potential pattern of disease or need for specific treatment.

For all restraint and cage handling procedures, individuals must have undergone training and been deemed competent prior to working unsupervised with animals.

Cage Environment and Stocking Density

Mice and rats must be kept in Individually Ventilated Cages (IVCs) unless there is a specific experimental reason not to do so. Ventilated caging is now an international standard of laboratory animal caging, it provides an improved atmospheric environment by actively removing ammonia build up in the cage and also provides greater protection for animal care staff from laboratory animal allergies. Different caging systems require approval in the relevant ANU Animal Experimentation Ethics Committee (AEEC) protocol.

ANU Research Ethics Office Animal Experimentation Ethics Committee Approved Document AEEC Approved Document_019_Standard_Laboratory Rodent Husbandry and Care V1.0 FINAL



Cages should be opened in a laminar flow cabinet or cage changing station wherever possible to protect the animal from pathogens that may exist in the environment. Where animals are being utilised for experiments that may pose a risk to the persons handling the animals this may be a Class II biological safety cabinet or cytotoxic approved cabinet. A risk assessment should be performed to indicate the appropriate class of cabinet. All cages must have a cage card which outlines the research group and protocol number, strain, sex, number of animals, date of birth or date(s) received, source and any interventions that have been undertaken.

Mice must be kept to a maximum of five post wean aged animals in a standard size mouse cage. A minimum of 60cm² must be available to each group housed animal as per the Victorian Code of Practice.

Breeding mice must be maintained as per AEEC Approved Document_018_Mouse Breeding Standards.

Environmental enrichment must be provided to all cages. A common way of delivering enrichment is through the use of nesting material mixed in with bedding. There are a range of enrichment materials available and the enrichment provided should be appropriate to the animal's age, life stage, strain, experimental purpose, number of animals in the cage, barrier status etc. Further advice can be provided by animal facility managers or the ANU vets. Individually housed animals should receive additional enrichment (e.g. tunnel, house, chew block) to mitigate the welfare impact of solitary housing. The intentional deprivation of enrichment for experimental reasons requires approval in the relevant AEEC protocol.

Room Environment

The temperature of animal holding areas for rodents must be kept between 19-24 degrees unless otherwise approved in an approved AEEC protocol.

Where possible, animal facilities related to ANU should maintain similar temperature ranges to avoid change stress in the animals.

Humidity should be aimed to be maintained between 40-70%.

Lighting should be on a 12 hour on/12 hour off cycle unless otherwise approved in the relevant AEEC protocol. Activity or equipment which causes light contamination during the dark period should be avoided as much as possible

Air flow should be maintained at 10-12 air changes per hour.

Noise and vibration for the general environment should always be kept to a minimum. Any spikes in these conditions should be investigated. Common issues may include nearby building work, loud equipment or excessively loud radios/music. Various methods can be employed to try to minimise the effect of unavoidable noise and vibration and these can be discussed with facility managers and the ANU vets.



It is a requirement that all animal facilities have temperature gauges that are alarmed beyond agreed parameters for that specific area. Any environmental problems in the room should be reported to the Animal Manager or Facility Coordinator immediately.

Sexing and Weaning

Animals must be sexed at weaning, or earlier, as a minimum to ensure the accurate division of animals to single sex cages. The sex of animals must be checked when animals are moved, when setting up breeding and before animals are issued for experimental or export purposes.

Animals must be weaned between 19-25 days of age. Late weaning may be considered on a case by case basis for particularly small animals but should be recorded appropriately to track for small/runted animals.

Weaning after 28 days of age increases the risk of unexpected litters from mating of mice with early sexual maturation and may increase fighting behaviour.

Late weaning can also increase the risk of new litters being lost and therefore close monitoring for the birth of additional litters is required.

Merging

Animals may be merged into shared cage environments on the following conditions;

- Animals must be the same sex
- Individual identification of each animal
- Males must be less than 4 weeks of age and must not be greater than eight days apart in age.
- Females of any age may be merged but must be monitored for signs of aggression. There may be specific strains where merging of females is not recommended.
- Stocking density restrictions must be maintained
- Cage cards and records must be updated to indicate merging.

Restraint

Where possible the ANU supports the use of low/non-contact restraint including the use of tunnels for examining and restraining animals.

Restraint may be required for a number of procedures and should be undertaken with consideration to the welfare of the individual animal.

Manual restraint should not result in undue stress and if the animal is obviously struggling for breath or significantly stressed the procedure must be stopped immediately and the animal monitored until fully recovered.

In barrier facilities, gloves must be disinfected with F10, ethanol or Virkon prior to opening the cage and again before handling any animals or contents of the cage as per the local area procedures.



Identification

The ANU supports the following identification methods for rodents:

- Ear punching (performed by adequately trained and competent staff)
- Temporary tail markings using permanent marker

The use of ear clips (unless animals have been imported from another facility) are not recommended. The use of tail tattoos and tail tipping must be specifically approved and justified in the relevant AEEC protocol.

Aged Animals

The holding of rats and mice beyond 12 months of age brings with it unique challenges including the increased chance of morbidity from spontaneous tumours, increased weight gain, poor condition and chronic weight loss.

These animals may require additional management to ensure that welfare is maintained including the use of a specific score card to monitor their wellbeing (see appendix 1).

The intended holding of animals beyond 12 months of age must have specific AEEC approval in the relevant protocol. If animals approach 12 months of age and you do not have specific approval to maintain them but they may be of value to your research, you should contact the ANU vets to discuss appropriate management and options relevant to their health status and your research requirements.

Breeding

Please refer to the AEEC Mouse Breeding Standards for breeding requirements for mouse models. Rat breeding should be undertaken with caution due to the limited use of rats at ANU however should it be needed, basic principles should be followed to reduce wastage and unnecessary proliferation of animals.

Provision of Food and Water

All rodents must have ad lib access to food and water unless specified in an approved AEEC protocol.

Where food or water restrictions are undertaken, gradual reduction in availability over a number of days should be undertaken as outlined in the approved AEEC protocol.

Food should be a standard rodent or breeding rodent chow and provided on a raised wire platform wherever possible to reduce the risk of soiling and to encourage normal behaviour. Food must be provided to ensure adequate turnover and should not be oversupplied to avoid risk of rancidity or mould growth. Food should be adequately decontaminated to reduce the risk of introduction of novel pathogens to animal facilities. This may be by irradiation or autoclave depending on the type of food and the facility. Any expired food should be discarded immediately.

Specific delivery of treated food (e.g. delivery of drugs or supplements) must be approved in the relevant AEEC protocol.

Water may be provided by a number of methods but should be checked for cleanliness and volume regularly.

Water provided in water bottles must be maintained above the sipper level when inverted in the tilted position in the cage (ie a minimum of 80 mls in most water bottle styles). This will ensure sufficient water is available for mice to be able to access the water through the sipper.

Sippers must be checked for risks of leaking and taken for service or removed from circulation if found to be leaking.

Monitoring, Intervention and Reporting

Animals must be visually checked daily. A more detailed check on all animals should be undertaken at least weekly. This may be more often for experimental animals or those expected to have disease progression as per the approved ethics protocol. A more detailed check includes inspecting each individual animal and will require disturbing the animals from the nest. Food and water must be topped up if not adequate at the check. Cages should be changed immediately if the water bottle has leaked into the cage.

It is expected that those caring for animals will have the skills and knowledge to provide immediate support for animals that may be found to be in any pain or distress.

All staff or research personnel approved to work with rodents independently must be competent in performing euthanasia of any animals that require immediate intervention.

Animals must be monitored as per the approved ethics protocol which may include the use of monitoring score cards. This should be checked against the specific conditions expected in the protocol and on the approved score system.

Where there is shared responsibility between animal technicians and research staff, the responsibilities of monitoring must be clearly defined and communicated between the groups to ensure that animal welfare is prioritised. This may include

- the need for meetings to discuss projects with an expected or unknown welfare impact
- agreed record keeping in the animal room such as a summary of the project, welfare risks etc
- agreed communication plan for unwell animals or adverse events (expected and unexpected)
- agreed responsibilities for checking of animals – where shared this should be well documented as to who takes responsibility for daily animal care checks and completing score sheets where applicable.



Animals that display unexpected illness or disease or die unexpectedly must be reported to the ANU Vets. This particularly applies to Unexpected Adverse Events (UAEs). For more information on UAEs refer to the University's procedure.

The ANU Veterinarians are also available for advice on all husbandry and animal care matters.

Mice with malocclusion must not be maintained and the trimming of teeth is not approved for any protocol. The technician may use their discretion as to the condition of the animal and may maintain an animal for a maximum of 24 hours to allow for collection of samples or sperm as may be appropriate for important animals.

Mice with hydrocephalus must not be maintained and must be euthanased on the same day as being found. If an animal is found to have hydrocephalus after the age of 3 weeks, it should be investigated as to the cause as this is an unusual finding.

Minimum Requirements

- Animals and their cage environment must be visually checked daily.
- Action must be taken if animals are found to be unwell or injured in any way. This may include intervention or supportive treatments or euthanasia where indicated. The ANU Vets should be contacted as per the relevant protocol requirements and monitoring systems.
- The cage environment and room environment must be maintained to set conditions and room temperature must be alarmed to an external alarm system.
- Animals must be weaned into separate cages of different sexes by 28 days at the latest unless there is specific ethics approval otherwise.
- Specialised breeding techniques, as defined in the AEEC Mouse Breeding Standards, and the holding of aged animals over 12 months of age must be specifically approved in an animal ethics protocol.
- Any changes to food or water provision that is not ad-lib and not standard rodent chow must be specifically approved in an animal ethics protocol.
- Mice with malocclusion must not be maintained and must be euthanased. Teeth trimming is not an approved procedure.
- Mice with hydrocephalus must be euthanased on the day of being found.

Appendices

Appendix 1 – Aged Rodents Score System

References and Resources

Fawcett, A. (2012) Guideline 22: Guidelines for the housing of mice in scientific institutions <https://www.animaethics.org.au/> (Accessed 24/08/2021).

ANU Research Ethics Office Animal Experimentation Ethics Committee Approved Document AEEC Approved Document_019_Standard_Laboratory Rodent Husbandry and Care V1.0 FINAL



NHMRC. Australian code for the care and use of animals for scientific purposes 8th Edition 2013 (Section 4.4.3) <https://www.nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes> [Accessed 7th September 2020]

Vaughan, L (2020) Flinders University safe work method: mouse – general husbandry version 8. <https://staff.flinders.edu.au/> (Accessed 20/08/2021).



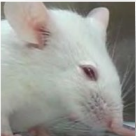
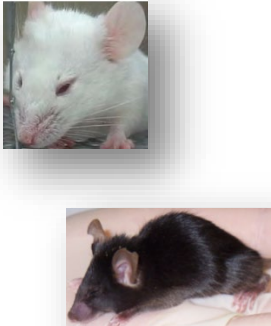
Victorian codes of practice for animal welfare. Code of Practice for Housing and Care of Laboratory Mice, Rats, Guinea Pigs and Rabbits <http://agriculture.vic.gov.au/agriculture/animal-health-and-welfare/animal-welfare/animal-welfare-legislation/victorian-codes-of-practice-for-animal-welfare/code-of-practice-for-the-housing-and-care-of-laboratory-mice,-rats,-guinea-pigs-and-rabbits> [Accessed 7th September 2020]

ANU Procedure for Managing & Reporting Unexpected Adverse Events <https://services.anu.edu.au/research-support/ethics-integrity/animal-ethics-policies-guidelines-and-forms> [Accessed 11th November 2021]

ANU Monitoring Score Card Template

<https://services.anu.edu.au/research-support/ethics-integrity/animal-ethics-policies-guidelines-and-forms> [Accessed 11th November 2021]

AGED MICE WELFARE ASSESSMENT

Score	Description	Action	Picture Examples
0	<p>Normal mouse in good condition (body condition score BCS 3/5). Coat smooth or possibly slight ruffle in aged mice without other signs. Good movement around cage or normal nesting behaviour. No grimace scale, mouse ears are forward, whiskers are exploring and eyes are open.</p>	<p>Mice over 12 months of age must be flagged and a score recorded weekly. Baseline weight to be taken once mouse at 12 months of age for comparison if deterioration occurs. No action needed</p>	
1	<p>Slower mobility when compared to younger animals of same strain. Still postures up to explore cage May begin to be mildly overweight or with more body fat in condition with hips/back bones difficult to feel. 10% body weight loss</p>	<p>Increase monitoring to weekly. Add weight monitoring which must be recorded weekly.</p>	
2	<p>Hunched and movement reduced even when cage opened in hood. Coat in poor condition and obviously progressing. May be obese in body condition or may begin to lose weight May be slightly dehydrated on skin pinch test. Only looks around cage with prompting. Breathing may be affected.</p>	<p>Increase monitoring to daily. Weight monitoring and recording to be increased to twice weekly. Check with researcher if mouse can be used sooner rather than held for long periods. Provision of pain relief or support treatment must be instigated if animal intended to be kept >24 hours</p> <ul style="list-style-type: none"> • Provide pain relief if indicated • Provide wet food on cage floor • Provide fluid support <p>Seek veterinary advice if animal to be maintained.</p>	
3	<p>Tumours are felt or seen either in body cavity or under skin surface. Weight loss may be marked with significant weight loss (>20% chronic weight loss from peak weight) and backbone protruding or significant weight gain to severe obesity where mouse is unable to move around cage freely. Grimace and other pain markers evident. Dehydration continues even with provision of wet food or other support. Poor movement around cage with indicators of pain associated. Swelling of joints that may be related to degenerative joint condition. Breathing is significantly laboured and mouse is obviously struggling. Abdominal breathing, rapid breathing noted.</p>	<p>Ethical cull</p> <p>Seek veterinary advice if research program has significant benefit for keeping animal.</p>	

Research Impact Assessment

Is the mouse expected to get sick? **Y** *Multiple age related conditions possible*

Are there known symptoms for illness? **Y** *See above score criteria*

If Yes – detail symptoms and progression in your specific score card. Remove unrelated/unnecessary score indicators from the template.

Are animals that are sick/show signs of inflammation/illness still able to provide you with quality research data? **Y**

Can the following supportive therapy be provided without impacting your research data? Where you identify specific treatments for your project these must be added to the “Action” in the welfare assessment template.

DRUG	POTENTIAL EFFECTS	ABLE TO USE?
Non-Steroidal Anti-Inflammatory Drugs (e.g. Metacam)	Reduce inflammation and therefore may not be suitable for some projects. Shouldn't be used with steroids.	Y / N
Fluid support (e.g. sterile saline/Hartmann's)	Minimal systemic effects, researchers should assess their own research and impact	Y / N
Steroid Drugs (e.g. Neocort)	These may impact the immune system and may not be suitable for some projects. Shouldn't be used with Non-steroidal drugs.	Y / N
Local Anaesthetic (e.g. lignocaine by cream - Emla or injection)	Minimal systemic effects, researchers should assess their own research and impact	Y / N
Trimming nails	Minimal effect unless studying dermatitis progression etc	Y / N
Food on floor of cage	No effect expected	Y / N
Recovery gel – with glucose etc	Minimal effects but does provide dietary change and may affect gut	Y / N
Opioids (e.g. Buprenorphine)	May impact some nervous system responses or other processes including respiratory depression	Y / N
Eye ointment (e.g. Conoptal)	Minimal systemic effects, contains antibiotic so should be assessed for project	Y / N
Tri-solfen (indicated for tail tipping/sample collection)	Contains lignocaine, bupivacaine, adrenaline and cetrimide. Minimal systemic effects but note adrenaline potential effect.	Y / N