



This document has been developed by The Australian National University's (ANU) Research Ethics Office. It has been endorsed by the ANU Animal Ethics Committee (AEC). 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.

## 019 Standard Laboratory Rodent Husbandry and Care V2.0

### 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.*

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## 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 “AEC” or Animal 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 Ethics Committee (AEC) protocol.

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 60cm<sup>2</sup> must be available to each group housed animal as per the Victorian Code of Practice.

Breeding mice must be maintained as per AEC Approved Document\_018\_Mouse Breeding Standards.

## **Environmental Enrichment and Social Housing**

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. As rodents are social animals, individual housing is not recommended. In exceptional circumstances such as isolation for welfare reasons, or for specially justified experimental reasons, individually housed animals should receive additional enrichment (e.g. tunnel, house, chew block) to mitigate the welfare impact. For females, a companion animal may be provided. All rodents which are individually housed for **any** reason should be the subject of veterinary consultation. Individual housing, and/or the intentional deprivation of enrichment for experimental reasons requires special justification and approval in the relevant AEC protocol.

## **Room Environment**

The temperature of animal holding areas for rodents must be kept between 18-24 degrees unless otherwise approved in an approved AEC protocol.

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

Humidity should be maintained between 40-70%.

Lighting should be on a 12 hour on/12 hour off cycle unless otherwise approved in the relevant AEC 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 10-15 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 should be weaned between 19-28 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.
- NB these conditions do not apply to setup of breeder cages.

## **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 for 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 AEC 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 AEC 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 AEC 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 AEC 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 AEC 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 AEC protocol.

Water may be provided by a number of methods, and should be checked for cleanliness and volume regularly. Where automated watering systems are used, these systems should be regularly checked and maintained.

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

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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 the PI's ultimate responsibility for animals on their protocols even when the animal care is being primarily undertaken by animal care staff. The AEC expectation is that researchers undertake regular checks on the welfare of the animals on their protocol (including breeders) and record this information, even when they are not actively being experimented on (excepting where facility access is restricted).

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, supportive treatments, or euthanasia where indicated. The ANU Vets should be contacted as per the relevant protocol requirements and monitoring systems.

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- 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 AEC 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.

## References and Resources

[Fawcett, A. \(2012\) Guideline 22: Guidelines for the housing of mice in scientific institutions](#)

[NHMRC. Australian code for the care and use of animals for scientific purposes 8th Edition 2013 \(Section 4.4.3\)](#)

[Vaughan, L \(2020\) Flinders University safe work method: mouse – general husbandry version 8.](#)

[Victorian codes of practice for animal welfare. Code of Practice for Housing and Care of Laboratory Mice, Rats, Guinea Pigs and Rabbits](#)

[Procedure for Managing & Reporting Unexpected Adverse Events](#)

[AEC Approved Document 009 Template Mouse Score System](#)

[AEC Approved Document 009a Template Aged Mouse Score System](#)

[AEC Approved Document 009b Template Mouse Body Condition Score](#)