

ANIMAL CARE AND USE STANDARD

The Animal Care & Use Standards are designed to provide guidance regarding good practice to institutional animal users and carers, as well as animal ethics committees, on the care and use of animals for scientific purposes such as research and teaching. The Standards are evidence-based, reflecting current or accepted good practice and allow for the flexibility that is required in research and teaching activities using animals.

HUMANE KILLING OF MICE AND RATS

This standard has been developed by the University of Melbourne Animal Care & Use Standards Committee, and endorsed by the University of Melbourne Animal Welfare & Ethics Committee.

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1. ASSOCIATED STANDARDS

This standard should be read in conjunction with the following University of Melbourne Animal Care & Use Standards:

- Anaesthesia of mice and rats
- Handling and restraining mice and rats
- Injections in mice and rats
- Training in non-surgical procedures

2. SUMMARY

- 2.1 Humane killing is the induction of death in an animal utilising methods that result in rapid loss of consciousness with minimum pain and distress. Humane killing may be done to avoid pain and distress (unexpected or defined humane endpoint), as a scientific endpoint, or for managing animal colonies (e.g. removal of animals of an inappropriate age or genotype).
- 2.2 Various methods of humane killing are available, each with inherent pros and cons. Selection of an appropriate method should involve consideration of: pain and distress, study requirements, equipment availability, animal age, and animal health status.

3. BENEFITS & RISKS

- 3.1 Selection of techniques that minimise pain and distress is critical for upholding the responsibility to optimise animal wellbeing.
- 3.2 Where pain and distress are expected or occurring, early humane killing can minimise any adverse animal experiences.
- 3.3 If humane killing methods are not performed by competent personnel then there is a risk of significant animal welfare compromise, particularly with physical methods.
- 3.4 Selection of the method for humane killing must address both the animal welfare and scientific objectives of the study.

4. PROCEDURE/PROTOCOL

4.1 Training

- 4.1.1 Trainees must be assessed as competent in handling and restraining the relevant species prior to commencing training in humane killing.
- 4.1.2 Humane killing must be performed only by people with appropriate training and experience and who are approved as competent. Training must include relevant anatomy, pain and distress, assessment of consciousness, assessment of death, use of equipment, monitoring/interventions for complications, and biosecurity relating to carcasses.
- 4.1.3 All trainees should undergo Office for Research Ethics & Integrity (OREI) online training on humane killing prior to commencing practical training (see section 6).
- 4.1.4 Competent trainers should provide the content, format and/or competency assessment sheets to the Animal Welfare Officer (AWO) prior to commencement of practical training. The AWO is also able to assist development of lesson plans and competency sheets. Competent trainers should have completed OREI online training on humane killing and been observed delivering training to an appropriate standard as determined by the AWO.
- 4.1.5 Where a humane killing procedure other than CO₂, sodium pentobarbitone, cervical dislocation, decapitation, or hypothermia (prior to 7 days of age) is proposed, the AWO must be invited to observe and provide advice on the humaneness of the technique.
- 4.1.6 Where training of physical methods is conducted, trainees must be assessed as competent to perform the procedure under deep anaesthesia on more than two consecutive animals prior to performing the procedure under supervision on conscious animals.
- 4.1.7 Where a trainee has performed a procedure that has not led to death the trainer must immediately humanely kill the animal and review the causes prior to continuing.

4.2 Preparation

- 4.2.1 Humane killing should be done in an area that prevents stressful auditory, visual and olfactory stimulation of other animals. The area should also be cleaned to remove animal debris and body tissue prior to use for humane killing.
- 4.2.2 Lighting, sound and handling of animals should be minimised prior to humane killing. Stimulation of animals can cause distress and lead to delayed loss of consciousness particularly with chemical methods.
- 4.2.3 Humane killing of animals with unexpected signs of pain and distress should occur in a room that prevents contamination of other animals with pathogens. Humane killing methods for animals with unexpected signs of pain and distress must comply with this Standard.

4.3 Prior anaesthesia

- 4.3.1 In many protocols animals are anaesthetised in order to collect tissues, perfuse, etc., before humane killing is performed. Non-recovery anaesthesia followed by humane killing is the preferred method whenever practical.

4.4 CO₂ Euthanasia

National Health and Medical Research Council (NHMRC, 2008) listing: recommended.

American Veterinary Medical Association (AVMA, 2013) listing: acceptable with conditions.

- The home cage should be used as the chamber for CO₂ gas wherever possible. The chamber must allow visualisation of animals that are inside.
- The flow meter needs to be calibrated and must ensure accuracy to within 5% of the chamber volume. The entry port for gas into the chamber should be located at the top in the middle to optimise mixing of CO₂.
- Mice and rats should be killed individually or as a familiar group in their home cage.
- CO₂ must be avoided in mice and rats under 10 days of age or those with respiratory compromise.

Method:

- 4.4.1 Take animal cage to CO₂ point in laboratory.

- 4.4.2 If only some animals in a cage are to be humanely killed, transfer these animals to a clean cage/chamber with paper towel on the floor.
- 4.4.3 The CO₂ flow rate must be set to 20% of the chamber volume per minute (acceptable range 15-25%).
- Observe the animals once CO₂ flow commences. They should not appear overtly distressed. Within 2 minutes the animals should become recumbent.
 - O₂ must not be administered to the chamber with CO₂.
- 4.4.4 Mice and rats must be kept in the chamber for at least 5 minutes or 1 minute beyond visible cessation of breathing.
- 4.4.5 Assess animal/s for loss of consciousness and indicators of death.
- Assess the animal immediately after removal from the chamber.
 - See section 4.9 of this Standard.
 - Examine the animals and equipment if the signs listed under section 4.9 are not present.
- 4.4.6 A secondary physical method of killing must be applied.
- Only commence after signs in section 4.9 are confirmed immediately prior.
 - See section 4.10 of this Standard.

4.5 Sodium Pentobarbitone

NHMRC (2008) listing: recommended.

AVMA (2013) listing: acceptable.

- Trainees must be assessed as competent in the relevant injection technique prior to beginning training in humane killing with sodium pentobarbitone.
- Commercial preparations of sodium pentobarbitone have a high pH and may cause discomfort if administered via routes other than intravenously. Where intravenous administration is available through a catheter then this route may be chosen. In the absence of a catheter intraperitoneal administration is often the preferred route for sodium pentobarbitone in rodents. Tail vein injection requires prior warming of animals and has a potential for perivascular injection which can be painful and reduce efficacy of the pentobarbitone.
- Prior intraperitoneal administration of other sedatives or anaesthetics may not constitute an animal welfare improvement as some of these solutions often have a low pH. There is limited published information comparing pain and distress associated with the various anaesthetic agents.
- Buffering of sodium pentobarbitone to reduce the pH is not recommended as it may lead to precipitation or reduced efficacy and delayed loss of consciousness. Buffer solutions must only be used if the pH has been tested and rapid loss of consciousness has been previously demonstrated. The AWO must observe and assess welfare impacts related to preparation, pH testing, solution stability at various time points and administration of sodium pentobarbitone at the start of all projects where buffering is proposed.

Method:

- 4.5.1 A dose of 150-200mg/kg sodium pentobarbitone must be administered either intravenously or intraperitoneally.
- 4.5.2 Assess animal/s for loss of consciousness and indications of death.
- Assess the animal 1 minute after visible cessation of breathing.
 - See section 4.9 of this Standard.
 - One repeat dose is allowable for animals if the signs listed under section 4.9 are not present after 5 minutes.
- 4.5.3 A secondary physical method of killing must be applied.
- Only commence after signs in section 4.9 are confirmed immediately prior.
 - See section 4.10 of this Standard.

4.6 Cervical Dislocation.

NHMRC (2008) listing: acceptable with reservations.

AVMA (2013) listing: acceptable with conditions.

- Training in cervical dislocation under deep anaesthesia should include additional methods for confirming death such as the use of a stethoscope or direct observation of the heart.
- Successful cervical dislocation should cause instant cessation of breathing.
- Due to the technical training requirements, prior anaesthesia should be considered or alternative methods such as CO₂.
- Cervical dislocation is not permitted in rats over 150 grams. Where cervical dislocation is proposed for rats under 150g, the age and expected weight range at the time of humane killing must be listed in the animal ethics application. The tail sheath of rats is prone to sloughing when held with the potential to cause considerable pain. Prior anaesthesia can mitigate pain caused by sloughing.

Method:

4.6.1 Pick the mouse or rat up (see: Handling and restraining mice and rats Standard).

4.6.2 Place the mouse or rat on the bench with the head facing away from your body.

4.6.3 Four methods are available depending on the size and strength of your hand.

Either:

- i) Use the thumb of your opposite hand to restrain the mouse against the back of the neck at the base of the skull **OR**
- ii) Use the thumb and forefinger of your opposite hand against the base of the skull with the flat part of your thumb and forefinger facing towards your body **OR**
- iii) Use the tip of your thumb on your opposite hand against the back of the neck at the base of the skull, with your 1st and 2nd fingers beside the mouse's head for support **OR**
- iv) Use a haemostat against the back of the neck at the base of the skull. Where haemostats are to be used the AWO must observe and assess the designated trainer/s for the project at least every two years. The model and brand of haemostat used by any personnel must only be those which the AWO has previously observed. Other objects such as pencils must never be used.

4.6.4 To produce the dislocation, quickly push forward and down with your thumb and forefinger/s (or haemostat) while with your restraining hand pull the mouse's body by the tail upwards and backward (approximately 30° angle). This action should be fast and applied with enough force to cause separation of the vertebrae from the skull.

4.6.5 The effectiveness of the dislocation can be verified by feeling for a separation of cervical tissues.

4.6.6 Assess animal/s for loss of consciousness and indications of death.

- Assess the animal immediately.
- See section 4.9 of this Standard.

4.7 Decapitation

NHMRC (2008) listing: acceptable with reservations.

AVMA (2013) listing: acceptable with conditions.

- Due to the technical training requirements, prior anaesthesia should be considered or alternative methods such as CO₂.

Method 1 - Scissors:

- Scissors must only be used on animals less than 7 days of age. The type of scissors (brand and model) being used must have previously been successfully tested on a dead animal. The sharpness of an individual pair of scissors must be tested prior to each day of use.

4.7.1 Pick up the animal and hold by body with the spine of the animal facing the scissors (see: Handling and restraining mice and rats Standard).

4.7.2 Place opened scissors gently around neck of the neonate or fetus.

4.7.3 Swiftly apply a firm single cut ensuring quick but careful decapitation (to cut through spinal cord as quickly as possible).

Method 2 - Guillotine:

- The type of guillotine (brand and model) being used must have previously been successfully tested on a dead animal. The sharpness of an individual guillotine must be tested prior to each day of use.

4.7.4 Place the animal's head in the guillotine with the path of the blade directed at the neck. Ensure fingers are not near the path of the blade.

4.7.5 Swiftly apply the blade as indicated by the guillotine model being used to cause decapitation in a quick single cut.

4.8 Fetuses and neonates

4.8.1 AVMA (2013) classified the following as acceptable (see references for detail):

- i) Euthanasia of the dam without removal of the fetuses.
- ii) Injectable barbiturates alone and in combination with local anaesthetics and anticonvulsants; dissociative agents combined with α_2 -adrenergic receptor agonist or benzodiazepines.

4.8.2 AVMA (2013) classified the following as acceptable with conditions:

- i) Inhaled anaesthetics.
- ii) Hypothermia prior to 7 days of age but ensuring no direct contact with ice or precooled surfaces.
- iii) Decapitation prior to 7 days of age.
- iv) Cervical dislocation. Care is required when handling mice and rats under 14 days of age by the tail because skeletal and connective tissues are weaker.

4.9 Loss of consciousness and indications of death

4.9.1 Death is difficult to confirm via external observation or manual palpation in mice and rats. A combination of methods should be used to ensure loss of consciousness including a firm toe pinch, lack of visible respiration, lack of digitally palpable heartbeat or respiration, grey mucous membranes and loss of the corneal reflex.

4.9.2 During training in methods performed under deep anaesthesia, the thorax may be opened after the relevant humane killing technique to directly observe cessation of a heart beat and assist in confirming death.

4.10 Secondary physical methods

4.10.1 As death is difficult to confirm following non-physical methods of humane killing, a secondary physical method must be applied. These methods must be performed immediately after assessment for loss of consciousness and indications of death (section 4.9). Methods used can include cervical dislocation (if under 150g), decapitation, bilateral pneumothorax, or surgical resection of either lungs or heart.

4.11 Carcasses

4.11.1 Carcasses must be made available for tissue sharing where possible.

4.11.2 Where an animal has unexpected pain or distress, or dies of a cause other than humane killing, the body must be preserved appropriately until a necropsy is performed. Appropriate personal protective equipment and decontamination must be adhered to as per the relevant facility procedures.

4.11.3 Carcasses must be disposed of immediately after humane killing, establishment of death and completion of any other scientific procedures. Disposal methods must prevent spread of pathogens or chemicals to animals and people.

5. MONITORING & INTERVENTION

5.1 Animals must be monitored closely throughout the entire duration of any humane killing procedures. Where anaesthetics are utilised, monitoring should conform to the following Standard: Anaesthesia of mice and rats.

5.2 Animals must be assessed for death immediately after procedures as per section 4.9.

5.3 The AFM and AWO must be notified immediately after killing any un-anaesthetised animals where a humane killing method is initially unsuccessful.

6. ADDITIONAL INFORMATION

- OREI online training: <http://orei.unimelb.edu.au/content/training-researchers>

7. ENFORCEABLE REQUIREMENTS

- 7.1 Performance of the procedure by competent investigators or trainees under the direct supervision of competent people.
- 7.2 Competency obtained in handling prior to undertaking training for humane killing.
- 7.3 For physical methods, trainees must be assessed as competent on more than two animals under deep anaesthesia before supervised training on conscious animals.
- 7.4 Calibrated flow meter to deliver 20% of chamber volume per minute for CO₂.
- 7.5 A secondary physical method must follow CO₂.
- 7.6 Use of the appropriate dose of sodium pentobarbitone as described above.
- 7.7 AWO to assess any proposed buffered solutions of sodium pentobarbitone
- 7.8 Cervical dislocation must not be used on animals greater than 150g.
- 7.9 AWO must observe any designated trainers from projects where haemostats are used for cervical dislocation at least every 2 years.
- 7.10 Use of scissors for decapitation only if the animal is under 7 days of age.
- 7.11 Assessment of scissor and guillotine prior to each day of use.
- 7.12 Adherence to monitoring described above.

8. EXEMPTIONS

Where adherence to this Standard conflicts with proposed work, the University's Animal Ethics Committees (AECs) may grant exemptions to all or part of the Standard. To seek exemption, applications should clearly outline how the proposed work deviates from the Standard, and justify the need for this. Before seeking exemption, it is recommended that you consult with the University's AWO.

9. UNEXPECTED ADVERSE EVENTS

An unexpected adverse event is any event, which impacts negatively on the wellbeing of animals, and which was not anticipated, or has occurred at a frequency or severity in excess of what was anticipated in line with the AEC approval. This can be a single or cumulative event, and will normally involve unexpected mortality, morbidity or injury. Anyone identifying an unexpected adverse event must act to remove and/or minimise any immediate risk to animals. Immediately thereafter, the University's AWO and relevant AFM must be notified of the event. The AWO will advise researchers of the appropriate response.

10. GLOSSARY

Scientific Term	Lay Description
auditory	hearing
conscious	aware of surroundings
olfactory	smell
perivascular	around the vessels
recumbent	lying

11. REFERENCES & RESOURCES

The following source material contributed to the development of this Standard:

- American Veterinary Medical Association. 2013. AVMA Guidelines for the euthanasia of animals: 2013.
- NHMRC. 2008. Guidelines to promote the wellbeing of animals used for scientific purposes.
- Newcastle Consensus Meeting on Carbon Dioxide Euthanasia of Laboratory Animals (9th August 2006).