

	Flinders University Safe Work Method Statement Methods of Humane Euthanasia in Mice 18/06/19		
			College of Medicine and Public Health Animal Facility
SWMS Number	RA Number		RA Score
SWMS- 1.4	RA- 1.4		MEDIUM
Contact Person	SWMS prepared by	AWC Approval Date	Review Date
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Legislation

- *Australian Code for the Care and Use of Animals for Scientific Purposes 8th Ed.*
- *Animal Welfare Act 1985*
- *Animal Welfare Regulation 2012*
- [Gene Technology Act 2000](#) (the Act)
- [Gene Technology Regulations 2001](#)
- *Work Health and Safety Regulations 2012*

University Policy

- Work Health and Safety Policy 2013
- Responsible Conduct of Research Policy 2016
- NHMRC Guidelines

Local Policy

Use of the College of Medicine and Public Health Animal Facilities by all staff and researchers of the College of Medicine and Public Health, Flinders University, is subject to awareness of, and adherence to the following:

Research Involving Animals:

- The University holds a licence for the use of animals for teaching and research purposes. To satisfy the requirements of the licence, anyone wishing to undertake teaching and research using animals must submit a proposal to the Animal Welfare Committee (via the Animal Ethics Review Sub-Committee). No work with animals may commence until written approval has been received from the Animal Welfare Committee. Standardised application forms for Research and Teaching can be found on the Flinders University website listed below. It is your responsibility to regularly check this site for updates to guidelines, forms etc.
http://www.flinders.edu.au/research/researcher-support/ebi/animal-ethics/animal-ethics_home.cfm
- **All staff and students involved in animal research must complete Animal Ethics Online Training (AEOT) and must also regularly attend Animal Researcher Information Sessions (ARIS).**

Safe Work Method Statement

Refer to Risk assessments, Safe Work Method Statement for chemicals, processes and plant equipment where appropriate. All projects must have an accompanying Risk Assessment signed by the Animal Facility Manager.

- SWMS 1.0 Mouse- Sexing, Handling, Restraint and Ear Notching
- RA 1.0 Mouse- Sexing, Handling, Restraint and Ear Notching
- SWMS 1.1 Mouse- Injection Techniques
- RA 1.1 Mouse- Injection Techniques
- SWMS 1.3 Mouse- Anaesthesia and Analgesia
- RA 1.3 Mouse- Anaesthesia and Analgesia

Personal Protective Equipment Required

- **Gloves**
- **Gown**
- **Mask**
- **Hair Net**
- **Shoe Covers**

Hazards and Controls

- **Animal bites- training, demonstrate competency, adhere to SWMS**
- **Animal Scratches- training, demonstrate competency, adhere to SWMS**
- **Needle Stick- DO NOT recap needles, dispose immediately into sharps containers, adhere to SWMS**
- **Chemical exposure- wear PPE and goggles**

Before Work Commences

Ensure that you are aware of the locations of the following:

- **Spill Kit**
- **Fire Extinguisher**
- **Eye Wash**
- **Exits**

Risk Assessment and SDS (Safety Data Sheet) - Ensure that you have read and understood for all the substances being used.

Equipment

- **Check for safety and electrical compliance**
- **Ensure that you have read and understood the Risk Assessment and Safe Work Method Statement**
- **Obtain training and demonstrate competency prior to procedure**

General Information

- **All procedures must be performed by trained competent staff listed on the Flinders University Animal Competency Skills Register.**
- **Training is available from senior animal facility staff or Animal Welfare Officer.**
- **Evidence of training is available in the “Staff Training Needs Analysis”.**

Humane Killing

Australian code for the care and use of animals for scientific purposes- 8th Ed- Section 3: Animal Wellbeing

3.3.45 The Method and procedures used for killing an animal must be humane and:

- (i) Avoid pain or distress and produce rapid loss of consciousness until death occurs,
- (ii) Be compatible with the purpose and aims of the project or activity,
- (iii) Be appropriate to the species, age , developmental stage and health of animal,
- (iv) Require minimum restraint of the animal,
- (v) Be reliable, reproducible and irreversible,
- (vi) Ensure that animals are killed in a quiet, clean environment away from other animals, and
- (vii) Ensure that death is established before disposal of the carcass, foetuses, embryos and fertilized eggs.

- (viii) Rodent fetuses are unconscious in utero and hypoxia does not evoke a sentient response. It is unnecessary to remove fetuses for euthanasia after the dam is euthanised.²

3.3.46 Dependent off spring of animals to be killed must be cared for or humanely killed.

NHMRC Methods of Humane killing and Euthanasia

Species	Recommended	Acceptable with Reservations
Mice	Inhalant- Carbon Dioxide Injectable- pentobarbitone sodium IP	Inhalant- Isoflurane Cervical Dislocation- animals Decapitation Exsanguination

Signs Indicative of Death

1. Absence of respiration - this is not sufficient as the heart may still be beating.
 2. Absence of Heart Beat - determined by palpation of the chest or stethoscope.
 3. Loss of colour in mucous membranes - no capillary refill after pressure is applied, mucous membranes become pale, dry, and sticky.
 4. Corneal and palpebral reflexes are lost - eye remains open, eye lids do not move when the eye ball is touched.
 5. Glazing of eyes - cornea appears opaque and dry.
- No one of the above signs is in itself reliable to determine the absence of life. **More than one sign must be observed** to confirm that death has occurred, or a secondary form of killing must be undertaken to confirm death.

Euthanasia by Carbon Dioxide - Ethical Considerations

- Administration of carbon dioxide has the potential to cause distress before loss of consciousness.²
- Inhalation of carbon dioxide at a concentration of 7.5% increases the pain threshold, and concentrations of 30% and higher cause deep anaesthesia and death with prolonged exposure. Mice show aversion and will forgo a food reward to avoid CO₂ concentrations of 15% and higher.^{2 & 8} An optimal flow rate of CO₂ in euthanasia systems should displace 10% to 30% of the chamber or cage volume per minute.²
- Concentrations >70% (pre-filling the chamber) causes rapid death, however, it irritates nasal, ophthalmic, and respiratory tract, causing discomfort and excitation.⁸

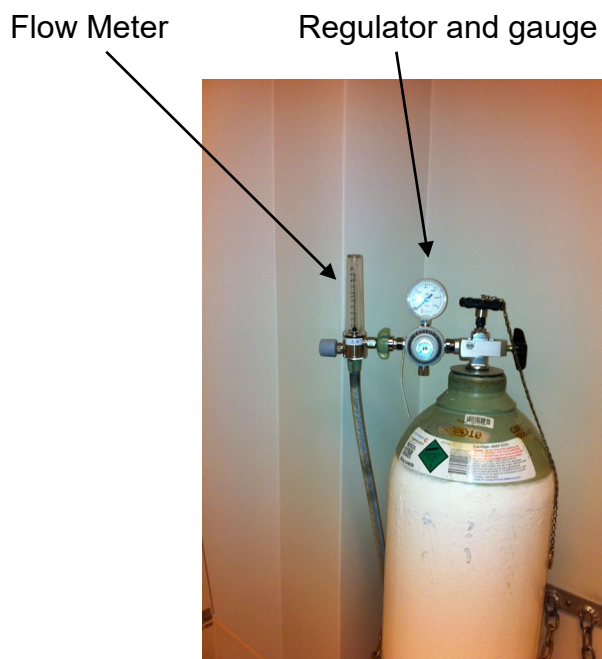
- Animals introduced to a pre-filled chamber experience 10-15 seconds of pain before they lose consciousness.⁸
- Animals should be anaesthetised before being introduced to concentrations of carbon dioxide above 30%.
- Once respiration ceases, the flow of CO₂ should continue for at least an additional four minutes to ensure that the animal is dead. If the animal is removed before the animal is confirmed dead, accidental recovery can occur when air is introduced. Death can be confirmed by a number of signs of death or a secondary form of euthanasia following CO₂ inhalation.
- Neonates are resistant to hypoxia. CO₂ asphyxiation is not recommended for mice pups less than 10 days of age.²

Euthanasia and Humane Killing by Carbon Dioxide

1. The Euthanasia chamber should be top loading with a close fitting (not air tight) clear lid to ensure that visibility is not obstructed. CO₂ is heavier than air and should be delivered at the base of the chamber. The CO₂ bottle should be fitted with a regulator and have a flow meter installed, allowing for greater control of the flow rate.
2. The chamber should have absorbent material on the bottom for urine absorption, but should not be thick enough not cover the CO₂ outlet.
3. Place animal in the chamber. DO NOT OVER CROWD THE CHAMBER. DO NOT PLACE LIVE ANIMALS INTO THE CHAMBER WITH DEAD ANIMALS.
4. One side of Euthanasia chamber is 52L, flow rate of 10.4 litres/min (20%) CO₂ is recommended to induce anaesthesia. If both sides of the euthanasia chamber are being used total chamber volume is 104L, use flow rate 14L/min (Max flow rate).
5. Once the animal becomes unconscious, the flow rate of CO₂ should be increased the up to 14 litres/min until respiration ceases. Allow the CO₂ to flow for a further minute, and then turn it off.
6. Leave the animal in the chamber for a further 5 minutes to ensure that death has occurred, and avoid accidental recovery. Confirm that the animal is dead before the body is disposed. (See "*Signs Indicative of Death*").
7. Records, including numbers and reason, must be kept of all animals humanely killed or euthanized.



(PHOTO: Euthanasia Chamber)



(PHOTO: Gas bottle, regulator and flow meter)

Cervical Dislocation

- Only personnel listed on the Flinders University Animal Competency Skills Register may undertake this technique without prior general anaesthesia of the animal. This procedure should not be used for large numbers of animals due to the potential for welfare compromise as a result of operator fatigue. Cervical dislocation may be used as a form of euthanasia from day of birth onwards.
 - Cervical dislocation may be used as an emergency form of euthanasia without premedication.
1. Place the animal on the bench. Restrain the dorsal neck skin with a thumb and forefinger behind the head while holding the base of the tail (*see photo*). Do not pull the tail but squeeze the cervical vertebrae immediately behind the skull in order to dislocate the atlanto-occipital joint. An instrument such as a scalpel handle or a 1mL tuberculin syringe can be used instead of thumb and forefinger for larger mice.
 2. The separation of the vertebrae will be felt. Vertebrae must be completely separated to ensure dislocation of the atlanto-occipital joint and the destruction of the brain stem. Palpate the vertebrae to ensure that the neck is broken.
 3. Confirm death before the body is disposed. (*See "Signs Indicative of Death"*).



(PHOTO: Cervical Dislocation)

Euthanasia by Barbiturate Overdose

- Sodium Pentobarbitone can be delivered via intra peritoneal (i.p.) injection.
- The i.p. route is the most practical, and is recommended by the NHMRC *Guidelines*, part III, Table H1¹. Pain may be associated with concentrated barbiturate solutions given via the i.p. route, but the degree of pain and the methods for controlling pain have yet to be determined.^{2, 11 & 12}
- Sodium pentobarbitone administered iv or i.p. is an excellent euthanasia agent; however, rodents should be placed in small cages to reduce excitement and injury during anaesthetic induction prior to death.¹¹
- The administration of pentobarbitone will result in residues in the carcass which may cause confounding variables in research outcomes.
- When injecting i.p., the irritancy of the barbiturate must be taken into account. Stock solutions need to be diluted consistent with anaesthetic grades of pentobarbitone sodium.
- Sodium Pentobarbitone needs to be diluted if administered by i.p. routes to a composition equivalent to Nembutal® Sodium Solution¹⁰ or with an equivalent volume of 2% w/v lignocaine hydrochloride. A concentration of 60 mg/ml is acceptable, as supported by the AWC and other ethics committees⁹. A dose rate of 180mg/kg will cause death. (See SWMS 1.1 Mouse Injection Techniques).

➤ Use the calculation below to work out volume to be injected:

$$\frac{\text{weight (kg)} \times \text{dose rate (mg/kg)}}{\text{Concentration (mg/ml)}} = \text{volume to be injected}$$

- Pentobarbitone 60 mg/mL administered by intra-peritoneal injection at the following dose volumes will be sufficient to ensure death:
 - 0.05 mLs for up to 5 grams bodyweight
 - 0.1 mL for 5 to 30 grams bodyweight
 - 0.2 mLs for over 30 grams bodyweight

- Confirm death before the body is disposed. (See “*Signs Indicative of Death*”).
- Records, including numbers and reason, must be kept of all animals humanely killed or euthanized.

Euthanasia from Birth to 7 Days of Age

- Mice in this category may be euthanized by decapitation. Use sharp scissors and in a single action decapitate by cutting through the high aspect of the neck.
- Gradual cooling may be used. Insert the pups into a plastic cup and lightly seal with a thin layer of tissue or cotton wool. Place this cup into a four degree refrigerator for 30 minutes. After this time, the pups may then be placed into ice-cold PBS for 5 minutes and then into a -18 degree freeze, or placed into liquid nitrogen, to confirm death.
- Cervical dislocation may be used by squeezing the neck behind the occiput of the cranium to dislocate the atlanto-occipital joint.

Euthanasia from 8 Days of Age

- Mice from 8 days of age onwards may be euthanised by cervical dislocation or intra-peritoneal injection, as previously described in this SWMS.

Other Methods of Euthanasia

- Euthanasia by exsanguination must be undertaken under general anaesthesia, and must be followed by a supplementary method of euthanasia, such as removal of all major abdominal or thoracic organs, the heart, or cervical dislocation.
- Decapitation as a form of euthanasia beyond 7 days of age requires ethical justification approved by the Animal Welfare Committee.
- Euthanasia by isoflurane overdose is acceptable provided it is within the confines of the AWC approval conditions of the specific project.

SWMS Review

This SWMS currently applies to the animals housed in the College of Medicine and Public Health Animal Facility. This SWMS will be reviewed 3 yearly, but also updated more frequently as policies, techniques and animal care requirements change.

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Useful References

1. NHMRC, *Guidelines to promote the wellbeing of animals used for scientific purposes*, Australian Government, 2008, <http://www.nhmrc.gov.au>
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5. http://www.flinders.edu.au/research/researcher-support/ebi/animal-ethics/animal-ethics_home.cfm
6. [http://www.medipoint.com/html/for use on mice.html](http://www.medipoint.com/html/for_use_on_mice.html)
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8. Wildlife Ethics Committee, (2013), *Euthanasia of research animals in the field policy*, Zoos SA, SA Museum, Government of South Australia.
9. Oak (2012) *MSDS: Nembutal® Sodium Solution (Pentobarbital Sodium Injection, USP) CII*, Oak Pharmaceuticals IL USA.
10. Harkness JE et al, (2010) *Harkness and Wagner's Biology and Medicine of Rabbits and Rodents*, 5th ed, Wiley-Blackwell.
11. Svendsen O et al, *Laboratory Animals*, 2007, 41, 197 – 203.

Any questions regarding the above guidelines and any technical advice/ assistance required can be directed to Animal Facility Manager.