

	Flinders University Safe Work Method Statement Methods of Humane Euthanasia in Rats 18/06/19			
				College of Medicine and Public Health Animal Facility
SWMS Number	RA Number		RA Score	
SWMS- 2.5	RA- 2.5		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 Regulations 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 2.0 Rat- Sexing, Handling, Restraint and Ear Notching
- RA 2.0 Rat- Sexing, Handling, Restraint and Ear Notching
- SWMS 2.1 Rat- Injection techniques
- RA 2.1 Rat- Injection techniques
- SWMS 2.3 Rat- Anaesthesia and Analgesia
- RA 2.1 Rat- 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.**
- **Training is available from senior animal house 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.

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
Rat	<p>Inhalant- Carbon Dioxide</p> <p>Injectable- pentobarbitone sodium IP at 180 mg/kg at a concentration of 60 mg/mL or less</p>	<p>Inhalant- Isoflurane^{bde} + cervical dislocation or cut diaphragm or remove vital organs or exsanguinate</p> <p>Cervical Dislocation^a</p> <p>Decapitation^{aeef}</p>
<p>Reservations:</p> <ol style="list-style-type: none"> a. Training required b. Work health and safety issues c. Inhumane d. Expensive e. Requires specialised equipment f. Aesthetically unpleasant 		

Signs Indicative of Death

- (i) Absence of respiration: This is not sufficient as the heart may still be beating.
- (ii) Absence of Heart Beat: Determined by palpation of the chest or stethoscope.
- (iii) Absence of reflexes: Righting, pedal withdraw, tail pinch, corneal and palpebral.
- (iv) Loss of colour in mucous membranes and periphery: Mucous membranes become pale, dry and sticky, and extremities become pale.
- (v) Pupillary dilation.
- (vi) Glazing of eyes: Cornea appears opaque and dry.

Examples of Indications for Euthanasia

- If the animal has reached the humane end point as described in the Clinical Record Sheet.
- Examples of indications for euthanasia:
 1. Amputation or crush injuries of limbs or tail.
 2. Self-mutilation.
 3. Broken limbs.
 4. Birthing difficulty.
 5. Rectal prolapse of > 5mm.

6. Generalised dermatitis.
7. Tail de-gloving injury (where skin on tail has been removed by picking up too far from the base of the tail).
8. Collapse, hypothermia, sepsis, severe dehydration, and non-responsive to human presence.
9. Emaciation (body condition score of 1 out of 5). For a description of body condition score criteria see Appendix 2.

Euthanasia by Carbon Dioxide - Ethical Considerations

- Carbon dioxide has the potential to cause distress in animals by pain due to the formation of carbonic acid on respiratory and ocular membranes, air hunger, a feeling of breathlessness and direct stimulation of ion channels within the amygdala associated with the fear response (AVMA, 2013).
- Rats show aversion to CO₂ levels between 15 to 20% (Wolfensohn and Lloyd, 2013, p 129) and are willing to forgo a palatable food reward to avoid exposure to CO₂ at concentrations of approximately 15% and higher (Neil and Weary, 2007).
- Carbon Dioxide has been used as a laboratory animal anaesthetic (Kohler et al, 1999).
- If an appropriate gradual displacement rate is used, such as 10 to 30% of the volume of the container per minute, commencing with normal ambient air, animals will lose consciousness before CO₂ concentrations become painful (AVMA, 2013).
- Concentrations >70% (pre-filling the chamber) causes rapid death, however, it irritates nasal, ophthalmic, and respiratory tract causing discomfort and excitation.
- Animals introduced to a pre-filled chamber experience 10-15 seconds of pain before they lose consciousness.
- Animals should be anaesthetised before being introduced to higher concentrations of carbon dioxide.
- A flow rate of 20% of the size of the chamber is recommended.
- Once respiration ceases, the flow of CO₂ may be increased and should continue for at least 5 additional minutes to ensure that the animal is dead.
- If the animal is removed before the animal is confirmed dead, recovery can occur when oxygen is introduced. Death can be confirmed via cervical dislocation following CO₂ inhalation.
- Neonates are resistant to hypercapnia- carbon dioxide is not a suitable euthanasia agent for rodent pups less than 10 days of age. Alternative methods such as cervical dislocation, decapitation, or chilling should be used.

Euthanasia and Humane Killing by Carbon Dioxide

- 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.
- The chamber should have absorbent material on the bottom for urine absorption, but should not be thick enough not cover the CO₂ outlet.
- Place the animal in the chamber. **DO NOT OVER CROWD THE CHAMBER. DO NOT PLACE LIVE ANIMALS INTO THE CHAMBER WITH DECEASED ANIMALS.**
- One side of Euthanasia chamber has a volume of 52L, the flow rate for this side of the chamber should be 10.4 litres/min (20% volume of the chamber per minute). If both sides of the euthanasia chamber are being used the total chamber volume is 104L, use a flow rate of 14L/min.
- Once the animal stops breathing, the flow rate of CO₂ may be increased to the maximum flow rate. The animal should remain in the chamber with a carbon dioxide flow, between 20 to 100%, for 5 minutes after the cessation of respiration.
- Confirm that the animal is dead before the body is disposed (*See Signs indicative of death*).
- Wipe and clean the container to ensure there are no body fluids remaining in the chamber prior to euthanizing more animals.
- Records, including numbers and reason for euthanasia, must be kept for all animals humanely killed or euthanized.



Euthanasia Chamber

Flow Meter

Regulator and gauge



Gas bottle, regulator and flow meter

Cervical Dislocation

- Animals must be anaesthetised before elective cervical dislocation unless the anaesthetic agent will interfere with the scientific outcome (see *SWMS 2.3 Rat Anaesthesia- Isoflurane*).
- Cervical dislocation must be performed by personnel competent in the procedure, and should not be used for large numbers to reduce fatigue and human error.
- Place the anaesthetised animal on the bench, and place a rod or scalpel handle behind the head. Push down with the instrument using both hands to separate the first cervical vertebra from the back of the cranium.
- The separation of the vertebrae will be felt. Vertebrae must be completely separated to ensure breakage of the spinal cord. Palpate the vertebrae to ensure that the neck is broken.
- Confirm death before the body is disposed (See *Signs indicative of death*).

Euthanasia by Barbiturate Overdose

- Sodium pentobarbitone at a concentration of 60 mg/mL or less can be delivered via intra peritoneal (i.p) injection.
- Commercial euthanasia solutions containing sodium pentobarbitone must be diluted if administered by the i.p route (a concentration of 60mg/mL or less is acceptable). A dose rate of 180mg/kg will cause death (see *SWMS 2.1 Rat Injection Techniques*).

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

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

- Confirm death before the body is disposed (See *Signs indicative of death*).
- Records, including the number of animals and reason for euthanasia, must be kept for all animals humanely killed.

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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Manager Animal Facility	Roxanne Collingwood	8204 4380 roxanne.collingwood@flinders.edu.au
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Useful References

American Veterinary Medical Association (2013), *AVMA Guidelines for the euthanasia of Animals: 2013 Edition*, AVMA, Schaumburg, IL, USA.

Kohler I et al (1999), Is carbon dioxide a useful anaesthetic for small laboratory animals? *Laboratory Animals*, 33 (2): 155-61

Niel L, Weary DM. Rats avoid exposure to carbon dioxide and argon. *Appl Anim Behav Sci* 2008; 114:572 – 581.

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Wolfensohn S and Lloyd M (2013), *Handbook of laboratory animal management and welfare*, 4th ed, Wiley-Blackwell

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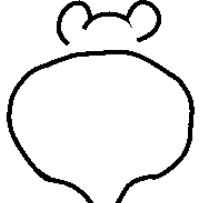
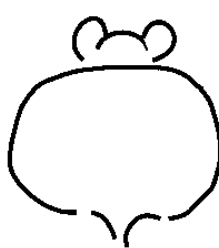
<http://www.adelaide.edu.au/ANZCCART/>

http://www.flinders.edu.au/research/researcher-support/ebi/animal-ethics/animal-ethics_home.cfm

http://www.medipoint.com/html/for_use_on_mice.html

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

Appendix: Body condition scoring criteria in mice

	<p>BC 1</p> <p>Mouse is emaciated.</p> <ul style="list-style-type: none"> ◦ <i>Skeletal structure extremely prominent; little or no flesh cover.</i> ◦ <i>Vertebrae distinctly segmented.</i>
	<p>BC 2</p> <p>Mouse is underconditioned.</p> <ul style="list-style-type: none"> ◦ <i>Segmentation of vertebral column evident.</i> ◦ <i>Dorsal pelvic bones are readily palpable.</i>
	<p>BC 3</p> <p>Mouse is well-conditioned.</p> <ul style="list-style-type: none"> ◦ <i>Vertebrae and dorsal pelvis not prominent; palpable with slight pressure.</i>
	<p>BC 4</p> <p>Mouse is overconditioned.</p> <ul style="list-style-type: none"> ◦ <i>Spine is a continuous column.</i> ◦ <i>Vertebrae palpable only with firm pressure.</i>
	<p>BC 5</p> <p>Mouse is obese.</p> <ul style="list-style-type: none"> ◦ <i>Mouse is smooth and bulky.</i> ◦ <i>Bone structure disappears under flesh and subcutaneous fat.</i>
<p><i>A "+" or a "-" can be added to the body condition score if additional increments are necessary (i.e. ...2+, 2, 2-...)</i></p>	

From: Ullman-Cullefé M.H, and Foltz J.F, 1999, Body condition scoring: a rapid and accurate method for assessing health status in mice, *Laboratory Animal Science*, Vol 49, No 3