

	Flinders University Safe Work Method Statement Rabbit – Humane Euthanasia 18/06/19		
			College of Medicine and Public Health Animal Facility
SWMS Number	RA Number		RA Score
SWMS- 4.7	RA 4.7		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 Statements for chemicals, processes and plant equipment where appropriate. All projects must have an accompanying Risk Assessment signed by the Animal Facility Manager

- RA 4.0 Rabbit- Sexing, Handling and Restraint
- SWMS 4.0 Rabbit- Sexing, Handling and Restraint
- SWMS 4.1 Rabbit injection techniques
- RA 4.1 Rabbit injection techniques
- SWMS 4.4 Rabbit Anaesthesia and Analgesia
- RA 4.4 Rabbit Anaesthesia and Analgesia

Personal Protective Equipment Required

- **Gown/ coat**
- **Mask**
- **Hair Net**
- **Shoe Covers**

Hazards and Controls

- **Animal bites- training, demonstrate competency, adhere to SWMS.**
- **Animal Scratches- training, demonstrate competency, adhere to SWMS.**
- **Animal Allergies- wear PPE when handling animals or cleaning pens.**
- **Needle – Do NOT recap the needle, dispose of needle in the sharps container.**

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

- **Ensure that you have read and understood the Risk Assessment and Safe Work Method Statement**
- **Obtain training before using any equipment**

General Information

- **All procedures are to 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 “Training Needs Analysis”.**

Humane Killing

Australian code for the care and use of animals for scientific purposes, 8th Edition, 2013 - 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
- (vii) Ensure that death is established before disposal of the carcass, fetuses, embryos and fertilized eggs.

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

NHMRC Methods of Humane Killing and Euthanasia

Species	Recommended	Acceptable with Reservations
Rabbit	Inhalant- None recommended Injectable- pentobarbitone sodium IP at 180 mg/kg at a concentration of 60 mg/mL or less	Inhalant- Isoflurane ^{bde}
Reservations:		
<ul style="list-style-type: none"> a. Training required b. Work health and safety issues c. Expensive d. Requires specialised equipment e. 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) Loss of colour in mucous membranes - no capillary refill after pressure is applied, mucous membranes become pale, dry, and sticky.
- (iv) Corneal and palpebral reflexes are lost - eye remains open, and eye lids do not move when the eye ball is touched.
- (v) Glazing of eyes - cornea appears opaque and dry.

Euthanasia by Barbiturate Overdose

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

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

❖ **Kits or small rabbits** can be euthanized by intraperitoneal injection.

- When injecting IP the PH of the barbiturate must be taken into account and may need to be diluted to ensure that it does not cause irritation.
- Sodium Pentobarbitone may need to be diluted if administered by IP routes, a concentration of 60mg/ml is acceptable.
- Dilute Lethabarb 1:6 (1ml Lethabarb 325mg/ml add 5 ml saline or sterile water to make a concentration of 54mg/ml), a dose rate of 180mg/kg will cause death.
 1. Gently restrain the kit and tilt backwards with its nose pointing towards the floor, this allows the internal organs to fall forward.
 2. Insert the needle in to lower left quadrant.
 3. Gently draw back on the plunger, no fluid should be drawn up into the hub of the needle, then inject the required dose.

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

❖ **Full size rabbits** should be euthanized via an IV injection of Sodium Pentobarbitone.

- Lethabarb does not need to be diluted if administered via IV injection as per SWMS 4.1 Rabbit injection techniques.
- Needle size 21 – 26G x 13-25mm, maximum bolus volume 1% of animal’s body weight.
- Anesthesia is not required although light sedation 0.1ml/kg s.c. ACP10 (Acepromazine 10mg/ml) is recommended.
 1. Carefully remove hair from the vein with a scalpel blade or clippers, and apply EMLA 5% lignocaine cream to the site at least 10 mins before procedure.
 2. The marginal ear vein can be dilated by one of several ways:
 - (i) Occluding the vessel with the thumb and forefinger.
 - (ii) Place the ear under a heat lamp for 3-5 minutes.
 3. The vein is immediately below the skin and must be entered at a very shallow angle, insert the needle bevel up almost parallel to the vein. The ear should be held taught and bent down while the vein is being entered at the point of the bend.
 4. Confirm death before the body is disposed (see “*Signs Indicative of Death*”).
 5. Records including numbers and reason must be kept of all animals humanely killed or euthanized.

Terminal Cardiac Bleed

1. Restrain and weigh the rabbit, then check the microchip identification number. Load 2 mLs of Lethabarb® into a 2 mL syringe and have it ready for step 14.
2. Administer acetylpromazine at 1 mg/kg, and/or xylazine at 5 mg/kg, by intramuscular injection, and wait for visible signs of sedation.
3. Then administer ketamine at 50 mg/kg (if given a premed of acetylpromazine only) or 35 mg/kg (if given a premed of xylazine with or without acetylpromazine) by intramuscular injection (opposite side to the side used for the premedication injection). Return the animal to a carry cage and wait for the onset of anaesthesia.
4. After 15 minutes, and when signs of anaesthesia are evident, check for evidence of surgical anaesthesia by observing the loss of the palpebral and pedal reflex.
 - **Palpebral reflex** – gently touch the inner corner eyelids of the rabbit’s eye, and when under anaesthesia, the rabbit should not blink.
 - **Pedal Reflex** – Squeeze the toes on one of the front paws, and observe any reaction.

5. If the rabbit blinks or withdraws its paw, wait for 5 - 10 minutes. Then if the rabbit still responds to these stimuli, either:
 - (i) Inject a further 0.15ml/kg of Ketamine (i.m.), and wait for another 5-10 minutes, then squeeze the toes again until you see no further reaction. If the rabbit fails to exhibit the desired anaesthesia depth, consult the Animal Welfare Officer.
 - Or,
 - (ii) Place the rabbit onto an anaesthetic mask (a non-rebreathing anaesthetic circuit using isoflurane between 1 to 3%, with an oxygen flow rate of 100 mLs/kg) and wait for evidence of surgical anaesthesia.
6. Place the animal into a dorsal recumbency (supine) position and feel for the rabbit's heartbeat. Insert a 19-gauge needle between the ribs where you can feel the heartbeat is the strongest.
7. While holding the needle, attach a 30ml syringe, with or without a three-way tap, drawing back the plunger slowly as it fills with blood (if no blood appears readjust the needle until blood fills the syringe). **Do not create an excessive vacuum in the syringe, as this will burst the red blood cells.**
8. When the syringe is full, leave the needle in the heart and remove the syringe.
9. Place blood from syringe into the desired collection container.
10. Re-attach syringe to the needle or three-way tap and repeat.
11. During the cardiac bleed, it is important to massage the heart continuously to obtain maximum blood volume.
12. If you cannot obtain any more blood from the rabbit and the heart is still beating, remove the needle and insert another, in the case of a blockage.
13. The minimum acceptable amount of blood allowed for a researcher is 80mls, however, more is preferable (above 100mls) depending on the size of the rabbit.
14. When you have obtained the maximum amount of blood from the rabbit, leave the needle in the heart (unless it is blocked), and inject 2mls of Lethobarb® into the heart.
15. Once death is confirmed, place the rabbit into an appropriate medical waste bag and dispose rabbit as medical waste.

Euthanasia by Terminal Cardiac Bleed with Saline/PBS Perfusion

1. Prepare two 50 mL syringes each containing either PBS or Normal Saline (one of these syringes should be attached to a 23 or 21 gauge needle, with or without a three-way tap). Ensure rat-tooth forceps, plain forceps, straight Mayo scissors, number 10 scalpel blade with number 3 handle, and 70% ethanol in a spray bottle are available.
2. Complete steps 1 to 5 for "Terminal Cardiac Bleed" (*above*). Step 3 (*below*) should not be commenced until a state of surgical anaesthesia is confirmed.
3. Apply a soaking cover of 70% ethanol to the ventral chest of the animal.

4. Place the animal into a dorsal recumbency (supine) position. Make two para-costal skin incisions the length of the thorax, and a transverse incision caudal to the costal arch. Identify the diaphragm and incise its entire length via another incision following the costal arch. Using Mayo scissors, cut the ribs in a para-costal manner, and remove the ventral rib cage and sternum.
5. Identify the right atrium of the heart, immobilise the apex of the heart with plain-tooth forceps, and incise the right atrium apex wall so that blood flows from the incision.
6. Puncture the apex of the left ventricle with a needle attached to the previously mentioned syringes, and start perfusing the PBS or saline. It may be necessary to hold the heart gently with blunt forceps in order to maintain the position of the needle.
7. Perfuse at the speed of 5 mL/minute if collecting the aorta for atherosclerotic plaque analysis. Ensure there is sufficient absorbent material, such as paper towel, to absorb the escaping blood and saline.
8. Perfuse until the liver bleaches and goes pale (unscrew the syringe and reload with a full one when the saline is exhausted, or reload the syringe and use the same hole in the ventricle to continue perfusing). Alternatively, if the needle is attached to a three-way tap, changing syringes is facilitated.
9. If the liver does not bleach, then the hepatic artery may have been cut or the syringe needle is not properly positioned in the ventricle.
10. Once adequate perfusion has been achieved, remove the syringe/needle, and harvest the organs and tissues. Cut the heart at its base and remove the entire organ to confirm death of the animal.

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.

Position	Name	Contact Details
Manager Animal Facility	Roxanne Collingwood	8204 4380 or 0415703 401 roxanne.collingwood@flinders.edu.au
Animal Welfare Officer	Dr Lewis Vaughan	0450 424 143 awo@flinders.edu.au

Useful References

<http://www.nhmrc.gov.au>

<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/home-1>

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

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

http://www.criver.com/files/pdfs/rms/nzw/rm_rm_d_nzw_rabbit.aspx

<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>

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