

 THE UNIVERSITY OF QUEENSLAND AUSTRALIA CREATE CHANGE	UQ Animal Ethics Committee - Standard Operating Procedure LAB_011 Euthanasia - Lethal Injection in Mice and Rats Institutional author: UQ Biological Resources AEC Reviewed & Approved: 17/09/2020	Version #3 Page 1 of 4
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LAB_011 Euthanasia - Lethal Injection in Mice and Rats

I. OBJECTIVE

To promote safe and humane euthanasia on mice and rats via intraperitoneal injection of sodium pentobarbital, as per Clause 3.3.45 of the Australian Code for the care and use of animals for scientific purposes.

NB: The use of (*) indicates this statement is dependent on the facility procedures

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II. COMMENTS / RECOMMENDATIONS

- Consideration must be taken as to the potential for stressful auditory, visual or olfactory stimuli that may be perceived by other animals. Efforts must be made to isolate these potential stressors:
 - Euthanasia or laboratory rodents should only occur in “terminal procedure rooms”(*),
 - Biosafety cabinets or fume hoods should be used for the procedure wherever possible,
 - Ensure the area is cleaned prior to use, and between animals,
 - Different species (i.e. rats and mice) should not be euthanised in the same area at the same time.
- Sodium pentobarbital will cause biochemical and histological tissue changes in the animal which has the potential to impact scientific findings – when planning to use this method, its suitability, relative to the desired scientific analysis, must be considered.
- Sodium pentobarbital has a relatively high pH (11-12) which should be expected to cause some level of irritation when injected intraperitoneal (IP) in rodents. Buffering of sodium pentobarbital is not recommended as it may lead to precipitation, reduced efficacy and potentially negative welfare outcomes.
- Sodium pentobarbital may be diluted as a means of refinement for practical reasons (by increasing the volume of the dose administered), however, it is unlikely to significantly reduce the pH of the final solution.
- If solutions other than water for injection or normal saline are mixed with sodium pentobarbital or administered IP immediately in succession, consideration must be made regarding stability (and efficacy) of the final solution. A UQBR veterinarian should be consulted.
- In laboratory rodents, when an intravenous catheter is in place sodium pentobarbital should be injected intravenous (IV) via the catheter, otherwise, sodium pentobarbital should be administered IP, as per this SOP.
- This procedure is appropriate for use in pregnant dams, and will result in the humane death of foetuses.

III. EQUIPMENT

- PPE*
Although PPE is facility dependent, minimum expectations include: disposable gloves, clean log-sleeved laboratory gown, hair bonnet, eye protection, face mask.
- Home cage - enclosure
- Needle & Syringe (as per LAB_028 Injections - Intra-peritoneal (IP) in Mice, Rats and Neonates)
- UQBR standard sodium pentobarbitone euthanasia solution (see V. Reference information for details)
- Cadaver bag

IV. PROCEDURE

- Retrieve the animal from the home cage and restrain it as appropriate for IP injection.

Conditions:

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2. Using the needle and syringe, inject 200-800mg/kg of sodium pentobarbitone IP (as per LAB_028 Injections - Intra-peritoneal (IP) in Mice, Rats and Neonates). This equates to:
 - a) Mice (<50g live body weight): 0.2mL of UQBR standard sodium pentobarbitone euthanasia solution
 - b) Rats (<500g live body weight): 2mL of UQBR standard sodium pentobarbitone euthanasia solution
See V. Reference information for details of UQBR standard sodium pentobarbitone euthanasia solution

If the animal's body weight is in excess of specified parameters (e.g. a 60g mouse), it is advised that you consult a UQBR veterinarian to reduce the volume of water for injection (or normal saline) used to make the dilution – ensuring the final dose administered is >200mg/kg sodium pentobarbital and the final volume injected appropriate for the species, as per LAB_028 Injections - Intra-peritoneal (IP) in Mice, Rats and Neonates.

3. Return the animal to the home cage and monitor for loss of righting reflex, followed by indicators of death (see V. Reference information for Indicators of death). If loss of righting reflex is not achieved within 2 minutes immediately repeat step 2 or implement an alternative method of humane killing in rodents e.g. CO₂, cervical dislocation (if body weight <150g), decapitation.

The righting reflex is the animal's ability to maintain a dorso-ventral position (when standing, sitting or lying down). Loss of the righting reflex is correlated with a loss of consciousness and sensory deprivation.

4. Once indicators of death are confirmed, collect the animal carcasses into cadaver bags and then seal the bag for disposal, else utilise the carcass for scientific purposes, as approved**.

If the animal has lost its righting reflex and withdrawal reflexes (e.g. toe pinch withdrawal), however, death has not been confirmed, a secondary method of euthanasia must be performed, for example CO₂, cervical dislocation, decapitation, bilateral thoracotomy, resection of the heart and or lungs, exsanguination and or cardiac perfusion.

V. REFERENCE INFORMATION

Table 1. UQBR standard sodium pentobarbitone euthanasia solution: dilution.

Volume of commercially available sodium pentobarbital (325mg/mL)	Volume of water for injection (or normal saline)	Final concentration of UQBR standard sodium pentobarbitone euthanasia solution
1mL	5mL	54mg/mL

Table 2. UQBR standard sodium pentobarbitone euthanasia solution: final volume for injection, relative to species (animals in excess of the weights listed may require dilution adjustment, see Procedure - Step 2).

Species	Standard volume to be injected IP per animal (this achieves a dose of 200-800mg/kg per animal)
Mouse (must be <50g body weight)	0.2mL
Rat (must be <500g body weight)	2mL

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Table 3. Indicators of death in laboratory rodents: all indicators must be observed to confirm death

Criteria	Confirmation
Absence of a corneal reflex	Place gentle pressure directly to the eyeball over the cornea. The eye lids should not blink. NB: The animal must be unconscious to perform this test.
Absence of spontaneous, rhythmic breathing	Deeply anaesthetised animals may exhibit shallow and irregular breathing, which must not be confused with a lack of spontaneous breathing. Thus, confirmation of a lack of spontaneous breathing requires astute monitoring and must not be used as sole criteria for confirming death.
Absence of a rhythmic heart beat	In rodents this may be assisted via observation of mucosal membrane colour, ECG or pulse oximetry, but it should be performed via direct thoracic palpation.
If there is any hesitation in confirming the above criteria a secondary method of euthanasia must be performed. For example CO₂, cervical dislocation, decapitation, *bilateral thoracotomy, *resection of the heart and or lungs, *exsanguination and or *cardiac perfusion. * Techniques that are not appropriate in a conscious animals – they require that the animal has lost its righting reflex AND withdrawal reflex (e.g. toe pinch withdrawal).	

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