LAB_100 Euthanasia Methods in Mice and Rats (Expiry November 2027)

You MUST also reference the individual SOP for the technique you are using when applying for animal ethics approval.

You must also read the individual SOP for the technique you are using when performing any euthanasia.

List of individual SOPs:

LAB _007 Euthanasia - Cervical Disclocation in Mice and Rats

LAB _008 Euthanasia - Carbon Dioxide Asphyxiation in Mice and Rats

LAB _009 Euthanasia - Decapitation in Mice and Rats

LAB _010 Euthanasia - Embryos and Neonates of Mice and Rats

LAB _011 Euthanasia - Lethal Injection in Mice and Rats

LAB _012 Euthanasia - Transcardial Perfusion in Mice and Rats

I. OBJECTIVE

This SOP provides the overview of currently used euthanasia techniques. **More detail on each procedure is provided in the individual procedure SOP.**

To describe the standard, safe and humane euthanasia of mice and rats used across UQ research projects, also reflecting the procedure used to train workers across UQ within UQBR.

To outline important information relevant to a technique. Shorter instructions on the specific steps to follow when performing the technique are provided in individual SOPs (see list above) to allow people undertaking a euthanasia technique to easily follow the important steps.

NOTE

- **When citing this SOP**
  - **You must also describe your chosen anaesthetic technique (or quote the relevant SOP you will be following)**
  - **You must also cite the relevant SOP for the specific technique**
- **The use of (*) indicates this statement is dependent on the facility procedures**
- **The use of (**) indicates this statement is dependent on AEC Approvals**

II. DEFINITIONS

Competent - "the consistent application of knowledge and skill to the standard of performance required regarding the care and use of animals. It embodies the ability to transfer and apply knowledge and skill to new situations and environments."[1]

The following two definitions are similar, and either can be used in most situations.

Euthanasia: - "The humane killing of an animal in the interest of its own welfare, to alleviate pain and distress".

Humane Killing: - "The killing of mice and rats using a humane method". For example, for culling or as part of a scientific investigation.

In animal ethics, the term **cull** generally means to remove animals from studies because they could not be used. For example were a sex or genotype that could not be used in the experiment. The term "cull" is also used when applying for animal ethics and reporting on outcomes. Ideally, the term "Cull" should not be used to mean Euthanise or "Humanely Kill".

As per the NHMRC Guidelines to promote the wellbeing of animals used for scientific purposes (2008), the key difference between humane killing and euthanasia is the reason that the animal is being killed. Humane killing is used at the end of studies to provide tissues for scientific purposes, when animals are no longer used for breeding and when stock are not required (e.g. unsuitable for particular research purpose).
Euthanasia refers to circumstances where pain, distress or suffering are likely to exceed humane end points and cannot be alleviated promptly. However, using the terms "euthanasia" and "humane killing" interchangeably is reasonable. The term "cull" should be reserved for describing animals that could not be used. These animals would be "humanely killed".

III. COMMENTS / RECOMMENDATIONS

- The humane killing of mice and rats MUST:
  - Result in a rapid loss of consciousness
  - Not allow recovery
  - Inflict minimal pain or distress
  - Be appropriate to the development stage of the animal
- If performed incorrectly, euthanasia can fail to kill the animal and cause pain and distress.
- In many protocols experimental animals are anaesthetised in order to collect samples, perfuse tissues or perform recordings before euthanasia is performed. Euthanising animals while under anaesthesia (without recovery) is the preferred method of euthanasia whenever practical.
- Using the restraint method that produces the least distress should always be used. Anaesthetising animals before performing other methods of euthanasia should be considered whenever possible.
- Death must be unequivocally established before disposal of carcase.
- This SOP includes the below methods of euthanasia for developmental stages as listed in the below table

[1] NHMRC, 2013, *Australian code for the care and use of animals for scientific purposes*, National Health and Medical Research Council (NHMRC).

Environment conditions

Animals must be killed or euthanised with efforts made to isolate them from potential stressful auditory, visual or olfactory stimuli that may be perceived by other animals. Therefore, animals are killed:

- In a quiet environment
- Separate from other housed animals.
- Not in the same room where rodents are permanently housed e.g. in terminal procedure rooms
- At different times for each species e.g. rats and mice are not euthanised in the same area and time
- In a location that can be cleaned between animals

Table 1. **Methods of euthanasia in rodents for developmental stage covered by this SOP.**

Methods of euthanasia in rodents for developmental stage covered by this SOP			
Method and detailed SOP reference	**Foetus (E10-E21) removed from uterus**	**Neonate (P0-P10)**	**Adult**
LAB_007 Euthanasia - Cervical Dislocation in Mice and Rats	X	✓	✓
LAB_008 Euthanasia - Carbon Dioxide Asphyxiation in Mice and Rats	X	✓	✓
LAB_009 Euthanasia – Decapitation in Mice and Rats	✓	✓	✓
LAB_010 Specifically Euthanasia via Hypothermia	All Embryonic ages	<P10	X
LAB_010 Specifically Euthanasia via Rapid Freezing	Following anaesthesia or Hypothermia acceptable from E15 – Birth.	Following anaesthesia or Hypothermia P0-P4.	X
LAB_010 Specifically Euthanasia via Rapid Freezing	<E15 Acceptable	X	X
LAB_011 Euthanasia - Lethal Injection in Mice and Rats	✓	✓	✓
Foetus not removed from the uterus – mammalian foetuses are unconscious in utero (hypoxia does not evoke a conscious response). Therefore, after a pregnant rodent has been humanely killed it is unnecessary to remove the foetus to effect humane euthanasia (of the foetuses).			

Table 2. **Indicators of death in laboratory rodents: all indicators must be observed to confirm death**

Indicators of death in laboratory rodents: all indicators must be observed to confirm death	
Criteria	**Confirmation**
Absence of eye reflexes	Absence of a corneal reflex: Place gentle pressure directly to the eyeball over the cornea. The eye should be unresponsive, and the eyelids should not blink. NB: This should only be performed in an unconscious animal. Deeply anaesthetised animals may lose corneal reflex so this cannot be used alone to confirm death. Additionally, the eyes and eyelids must be unresponsive, and the eyes should appear "glazed" with fixed-dilated pupils.
Absence of spontaneous, rhythmic breathing	There is a complete lack of breathing and respiratory movements. 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 heartbeat	Asystole is confirmed via direct thoracic auscultation or palpation. i.e. a finger and thumb placed either side of the heart cannot detect a heartbeat. This judgement may be assisted via observation of mucosal membrane discolouration, absence of ECG or pulse oximetry conduction.
If there is any hesitation in confirming the above criteria a secondary method of euthanasia must be performed. For example, lethal injection, decapitation, *bilateral thoracotomy, *resection of the heart and or lungs, *exsanguination and or *cardiac perfusion. *Indicates techniques that are not appropriate in a conscious animal – they require that the animal has lost its righting reflex (e.g., unconscious, lying on its side) AND withdrawal reflexes (e.g., toe pinch withdrawal).	

IV. SAFETY AND COMPLIANCE

- Possible risks include mouse bite injury, spills, exposure to infectious agents and psychosocial harm.
- The person undertaking this task must ensure all relevant approvals are in place, training has been undertaken and risk assessments have been performed. If unsure, consult your supervisor.
- Carbon dioxide should never be used without adequate ventilation or airflow
- All UQBR facilities are fitted with CO2 timers designed to turn off after a pre-determined period. The purpose of these is to minimise the risk of incidental CO2 exposure.
- All UQBR facilities CO2 euthanasia stations are fitted with a regulator to restrict the flow of gas
- Facility protocols should be followed.
- All UQBR facilities will display the signage 'UQBR-REF-033 Use of Euthanasia Stations' next to the euthanasia station to support the consistent proper use of CO2 euthanasia stations.
- UQBR risk assessments relevant to this task include:

UQSafe Reference	Risk Assessment Name
1128	PPE Requirements – Exposure to laboratory animal allergens (LAA)
7005	Manual handling
3657	Handling and restraint of laboratory animals under 10kgs
10439	Wellbeing in laboratory animal workers

V. TRAINING CONSIDERATIONS

Any training on live animals must be part of an approved process or have the training activity approved in an animal ethics project.

- All animal euthanasia and humane killing MUST:
  - Be performed by appropriately trained personnel
  - Who have been deemed to be competent in the procedure.
  - Are confident in completing the procedure
  - OR be under the direct supervision of a person who is competent

- Training in euthanasia MUST:
  - Be undertaken on cadaver animals until competent
  - Further training should be undertaken on animals under general anaesthesia
  - Training should include additional methods for confirming death such as the use of a stethoscope or direct observation/palpation of the heart *
- 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 heartbeat and assist in confirming death.
- Workers trained by UQBR must complete:
  - Pre-requisite training such as handling and restraint

- Relevant online learning prior to receiving training in euthanasia procedures

VI. EQUIPMENT

- Refer to UQBR-REF-033 CO2 Euthanasia Stations for instructions on how to use timers.

VII. PREPARATION

- Check AEC approvals to ensure that the correct procedure and personnel are approved for the planned work
- Check cage cards, animal records and identification to ensure the correct animals are euthanised.

VIII. TECHNIQUE CONSIDERATIONS

LAB_008 Euthanasia - Carbon Dioxide Asphyxiation in Mice and Rats

- The home cage is the preferred location to deliver the CO2 to minimise stress associated with handling and environment changes.
- The rate of carbon dioxide (CO_2) exposure to rodents (i.e. the flow rate) must be controlled using a flow meter and a regulator, these are fitted to each UQBR CO2 station.
- Cage volumes in *UQBR-REF-033 Use of Euthanasia Stations* have been digitally measured at the UQ Science Workshop. This accurate cage volume supports confidence in the calculation of the flow rate to use for each cage type.
- This technique is appropriate for use in pregnant dams and will result in the humane death of foetuses.
- Low CO_2 flow rates, relative to chamber volume (e.g. <30% displacement/minute), MUST not occur because they will result in a prolonged periods of respiratory distress (i.e. “air hunger”). Therefore, it is important to know the delivery chamber volume and to use a flow regulator.
- Placing rodents directly into “pre-filled” high concentrations of CO_2 (e.g. >90%) MUST NOT occur because it will cause pain to the animals. Therefore, ensure any waste gas is discarded when cleaning the chamber between animal groups.
- If pooling rodents is required due to workflows or a high volume of rodents to euthanise the following measures are followed:
  - Sexes are not mixed
  - No more than 2 cages at maximum density are pooled

Table 3. Guiding information on CO2 flow rates in rodents.

Guiding information relative to CO_2 flow rates in rodents.

- The rate of carbon dioxide (CO_2) exposure to rodents (i.e. the flow rate) must be controlled.
- CO_2 flow rate should be gradually administered at 30% of the chamber volume displacement per minute. [It is noted that some recent literature supports a much wider range of acceptable CO_2 flow rates, however, the number of studies and the limitations of their findings does not provide sufficient evidence to be confident of the associated welfare impact to rodents]. This is within best practice (AVMA 2020). Only those strains or models that have an identified abnormal response to 30% flow rate may apply a range of 20-40% flow rate as advised by a UQBR Veterinarian.
- To calculate the flow rate (Chamber volume in Liters) x (0.3 or 30% displacement) = (Flow rate L/min).
- The required rate to set the flow meter when using UQBR Euthanasia stations is outlined below

Type of carbon dioxide delivery chamber used		Minimum CO_2 flow rate to ensure ≥20% chamber volume displacement/ minute
Tecniplast Greenline Mouse IVC		**Volume 8.45L at 30% Displacement = 2.5L/minute**
OptiMICE cage		**Volume 10L at 30% Displacement = 3L/minute**
OptiRAT cage		**Volume 23.3L at 30% Displacement = 7L/minute**
Tecniplast Greenline Rat IVC		**Volume 35.3L at 30% Displacement = 10.5L/minute**
Tecniplast Greenline Rat IVC		**With Tecniplast lid – 4L/minute Volume 46.6L at 30% Displacement = 14L/minute** **Without lid (flat custom) Volume 30.2 at 30% Displacement = 9L/minute**

Carbon Dioxide – Neonates

- Sealed bags may be used as an alternative option to the home cage or specific euthanasia chamber
- Once the chamber is filled to 100% CO_2, neonates must be left undisturbed within the high concentration gas chamber for a prolonged period, compared to adults as listed in the table below.

Minimum time of 100% carbon dioxide exposure to confirm death in mice and rats		
AGE	**MICE**	**RATS**
Non-haired pups 0–6 days	60 minutes	40 minutes
Haired pups, eyes closed 7–14 days	20 minutes	20 minutes
Haired pups, eyes open, pre-weaning 15–20 days	10 minutes	10 minutes
Weanlings and adults 21+ days	5 minutes	5 minutes

LAB_009 Euthanasia – Decapitation in Mice and Rats

- In adults, although decapitation is often considered aesthetically unpleasant, when performed correctly, this procedure should be considered comparable to cervical dislocation in terms of welfare impact to the animal.
- Scissors and guillotines must have sharp blades and must have recently proven that they are fit for purpose (by prior use on cadavers). Scissors must be cleaned of debris between animals – this helps to reduce stress in rodents being handled and helps to maintain good working order of the scissors.
- In pups, this procedure is generally considered the most reliable (and thus humane) euthanasia method

LAB_007 Euthanasia - Cervical Dislocation in Mice and Rats

- An instrument (scalpel handle, pen) is not used to hold the head without prior approval from the AEC**
- Historically the language used in this SOP has created a consistent dialogue amongst workers as to the ‘best’ technique for cervical dislocation. It is widely accepted that there is some variation at this step of the procedure e.g. push vs pull on the head, whether the movement should be horizontal vs the tail being held upwards etc. The literature does not clearly suggest a benefit of one method over the other. Therefore, provided the outcome of a rapid death with complete separation of the spinal cord from the skull is achieved, these minor variations are acceptable.
- This SOP applies to mice and small rats (less than 150 g).

Species and size of rodents covered by this SOP		
AGE	**MICE**	**RATS**
Neonate	✓	✓
Adult	✓	< 150g only under anaesthesia As a secondary confirmation of death in UQBR ✓
Adult	-	> 150 g X

LAB_011 Euthanasia - Lethal Injection in Mice and Rats

- 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.
- This procedure is appropriate for use in pregnant dams and will result in the humane death of foetuses.

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**
1mL	5mL	54mg/mL

UQBR standard sodium pentobarbitone euthanasia solution: Final volume for injection relative to species	
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
Mouse > 51g	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
Rat > 501g	

LAB_010 Euthanasia – Hypothermia and Rapid Freezing in Mice and Rat Embryos and Neonates

- Mammalian foetuses are unconscious in utero due to a combination of factors, such as low oxygen tension and hormonal influences in the uterus suppressing consciousness.
- Rat and mouse pups are born neurologically immature and their afferent pain pathways are not well developed until after 5-7 days old.
- There is no data to support the use of hypothermia as a single method, and it should always be followed with a secondary method following loss of movement (e.g. decapitation).

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Version #	Reviewing AEC (note: all other relevant AECs ratify the approval)	AEC Review Date	Approval To Date
[#]		[DD/MM/YYYY]	

Conditions:

- Investigators named in an animal ethics application, relative to this SOP, must be competent to implement the SOP
- Any variation to this SOP must be described in the relevant animal ethics application
- If this SOP has not been reviewed and approved by a UQ AEC within the last three years it is no longer valid and cannot be used in animal ethics applications until reapproved (see “AEC Reviewed/Approved” date in this document’s header).