# LAB_007 Euthanasia-Cervical Dislocation in Mice and Small Rats (Expiry: May 2027)

## I. OBJECTIVE

To describe the standard, safe and humane use of cervical dislocation for euthanasia of mice and small rats (less than 150 grams) used across UQ research projects, also reflecting the procedure used to train workers across UQ within UQBR.

**NOTE for citing this SOP in ethics applications: If you plan to use anaesthesia for mice and when applying to euthanise rats, you must also describe your chosen anaesthetic technique (or quote the relevant SOP you will be following)**

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

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

**"Humane Killing"**: - "The killing of mice and rats for culling or as part of a scientific investigation"

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.

## III. COMMENTS / RECOMMENDATIONS

- The humane killing of mice and rats must result in a rapid loss of consciousness without recovery, and must inflict minimal pain or distress.
- If performed incorrectly, cervical dislocation can fail to kill the animal and cause pain and distress.
- All animal euthanasia must be performed by appropriately trained personnel who have been deemed to be competent in the procedure.
- This procedure should only be performed by confident individuals.
- Animals must be killed in a quiet environment wherever possible.

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

- Animals should be killed in a location separate from other housed animals.
- Euthanasia procedures should not be performed in the same room where rodents are permanently housed.
- Death must be established before disposal of carcase.
- Methods of killing must be appropriate to the developmental stage of the animal.
- This SOP applies to mice and small rats (less than 150 g).
- The use of an instrument (scalpel handle, pen) to hold the head is not included in this SOP so should not be performed without prior approval from the AEC. Please include the use of an instrument as a deviation from the approved SOP if you want to use an instrument. UQBR training will not include the use of an instrument to hold the head unless prior approval has been obtained.
- In many protocols experimental animals are anaesthetised in order to collect samples, perfuse tissues or perform recordings before euthanasia is performed. This method of anaesthesia without recovery is the preferred method of euthanasia whenever practical.

**Table 1. 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.<br>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.<br>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. when a finger and thumb placed either side of the heart cannot detect a heartbeat.<br>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 in deeply unconscious animals. These include, lethal injection, decapitation, **resection of the heart and or lungs, exsanguination and or cardiac perfusion.**<br>**Techniques in bold**-Indicates techniques that must be performed in a **deeply unconscious animal** – they require that the animal has lost its righting reflex (e.g., unconscious, lying on its side) AND all 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.
- Facility protocols should be followed.

## V. TRAINING CONSIDERATIONS

- All animal euthanasia must be performed by appropriately trained personnel who have been deemed to be competent in the procedure.
- Training for cervical dislocation must be undertaken on cadaver animals until competent.
- Further training should be undertaken on animals under general anaesthesia
- Training in cervical dislocation under deep anaesthesia 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.

## VI. EQUIPMENT

- PPE*
- Cadaver bag*
- Bench protector material
- Anaesthesia equipment*

## 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. PROCEDURE

**Cervical Dislocation of Mice**

1. Prepare the workspace
   *Use clean bench coat or a wiped area. Select the appropriately sized cadaver bag.*

2. Select the correct animal from the cage and place onto your work area, refer to LAB_006 Handling and Restraint in Mice and Neonates
   *Check the animal identification, remove the mouse onto your working space*

3. Place the animal on a flat firm surface, ventral (belly) side down.

4. Immobilise the mouse by using your hand to grasp the base of the tail with your forefinger and

thumb. Place your remaining fingers on the hind quarters of the animal to immobilise the mouse.

5. Use the thumb and forefinger of your other hand against the base of the skull as shown in 2 different hand placement methods in Figure 1.
   *Hold firmly in place. See Figure 1 for example.*

6. To produce the dislocation, quickly push or pull (depending on hand placement) forward and down with your thumb and forefingers, with your restraining hand stabilising the tail end of the mouse's body by the tail upwards (approximately 30° angle).
   *This action should be fast and applied with enough force to cause separation of the vertebrae from the skull. This will cause a separation of the spinal cord from the skull resulting in immediate death. The skin may be intact but there should be an obvious visual and palpable separation of the skull from spinal column and separation of the cervical tissues. Muscular contractions may be observed post death. This SOP describes a standard technique where the tail is not pulled, but simply held for restraint.*

   *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.*

7. Verify death by confirming separation of the spinal cord from the skull in addition to other signs of death (see Table 1)

8. Ensure all record keeping has been accurately undertaken.
   *The person euthanising or humanely killing the animal is responsible for recording all details following animal facility procedures (i.e. cage card, movement log, score sheet)*

9. The person euthanizing or humanely killing the animal is responsible for correct disposal of the carcass.
   *If necropsy is required, place the fully labelled carcass in the fridge (not freezer) and follow procedures in LAB_022*

**Figure 1. Example of finger placement so the hand movement would be a "push" (Left) or pull (right).**

**Considerations for cervical dislocation in rats**

- This procedure may only be performed in rats <150 g following specific justification that has been accepted by a UQ AEC
- Cervical dislocation in rats may be used within UQBR as a secondary confirmation of death (assuming the above conditions are satisfied)

**Cervical Dislocation of Small Rats < 150 g (only under anaesthesia)**

1. Anaesthetise the rat, ensuring conscious responses have been ablated
   *Rats must be anaesthetised using the anaesthesia protocol approved by the AEC in your application*

2. Grasp the tail with the dominant hand. Place the opposite hand over the top of the rat's head and firmly grasp around the neck with your thumb and forefinger
3. With a quick motion forcefully push/pull to dislocate the neck from the spinal column
   - This will cause a separation of the spinal cord from the skull resulting in immediate death.
   - The skin may be intact but there should be an obvious visual and palpable separation of the skull from spinal column.
4. Repeat this motion 2-3 times to ensure successful dislocation
5. Confirm successful dislocation by feeling for complete separation on the sides of the neck and the ability to extend the head backwards with ease

## IX. BIBLIOGRAPHY

1. J Am Assoc Lab Anim Sci. 2012 May; 51(3): 352–356. Published online 2012 May. PMCID: PMC3358985.PMID: 22776194 Assessing Cervical Dislocation as a Humane Euthanasia Method in Mice Larry Carbone,1,* Elizabeth T Carbone,1 Elizabeth M Yi,1 Diana B Bauer,1 Krista A Lindstrom,1 John M Parker,1 Jamie A Austin,1 Youngho Seo,2 Anisha D Gandhi,3 and James D Wilkerson1

2. National Health and Medical Research Council (NHMRC) 2008, Guidelines to promote the wellbeing of animals used for scientific purpose, viewed 11 April 2019, https://www.nhmrc.gov.au/about-us/publications/guidelinespromote-wellbeing-animals-usedscientific-purposes

3. AVMA. (2020). AVMA guidelines for the euthanasia of animals: 2020 Edition. American Veterinary Medical Association (AVMA) Retrieved from https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf

| Version # | Reviewing AEC<br>(note: all other relevant AECs ratify the approval) | AEC Review Date | Approval To Date |
|---|---|---|---|
| 7 | | May 2024 | |
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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).