

**University of Prince Edward Island
Animal Care Committee
Standard Operating Procedure**

SOP #: ACC - CT01

SOP Title: Rodent Euthanasia

SOP Section: Clinical Technique

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1.0 Introduction

- 1.1 Ensure that all individuals responsible for euthanasia are appropriately qualified, trained and adhere to UPEI Animal Care Committee-approved protocols and policies.
- 1.2 Maintain equipment to ensure optimal performance.

2.0 Materials

- Euthanasia chamber
- CO₂ in a compressed gas cylinder
- Barbiturates/euthanasia solution
- Appropriately sized needles and syringes [i.e., 23 and 25 G needles, 1 cc syringe(s)]
- Decapitation device (i.e., guillotine) or dedicated scissors
- Sharps container
- Bag or container for animal carcass disposal

3.0 Procedures

3.1 Non-physical Methods

- i. CO₂ asphyxiation

Note: This method is not approved for neonates up to 10 days of age.

1. Place the lid connected to the CO₂ tank on the cage containing the animal(s). Do not overcrowd the chamber. Use a chamber large enough to permit each animal to stand on the floor of the chamber with all four feet and have sufficient space to turn around and perform normal postural adjustments. (~ 100 cm²/mouse) (~ 500 cm²/rat).
2. Open the valve and set the flow so to displace at least 20% of the chamber volume per minute (usually ~5 L/min) to induce rapid unconsciousness with minimal distress to the animals.

3. Maintain gas flow for at least 1 minute after respirations have ceased.

Important: Verify that an animal is dead before removing it from the chamber by making sure there is no respiratory movement for at least 3 additional minutes.

4. If the animal is not dead (or for additional security), follow the CO₂ narcosis by another method of euthanasia (for instance, cervical dislocation).

ii. Overdose of inhalant anesthetic

1. Expose the animal to a high gas concentration using an anesthetic vaporizer or soaked gauze in a closed glass container.
2. Vapors are inhaled until respiration ceases for at least 1 minute and death ensues.

Important: Verify that an animal is dead before disposing of the carcass, by making sure there is no respiratory movement for at least 3 additional minutes.

3. If the animal is not dead (or for additional security), follow the anesthesia by another method of euthanasia (for instance, cervical dislocation).

iii. Overdose of injectable barbiturate

1. Inject Pentobarbital solution at a dose of at least 100mg/kg IV or IP.

Important: Verify that an animal is dead before disposing of the carcass, by making sure there is no respiratory movement for at least 3 minutes.

2. If the animal is not dead (or for additional security), follow the injection by another method of euthanasia (for instance, cervical dislocation).

3.2 Physical methods

Considerations

1. Use these techniques only when scientifically justified by the user and approved by the UPEI Animal Care Committee.
2. Apply prior anesthesia or sedation whenever possible.
3. If anesthesia is contraindicated, these methods can be applied only by a demonstrated skilled and experienced person. Training provided by the University Veterinarian.

i. Cervical dislocation

Note: This method can be applied to rodents weighing less than 200 g (if not under deep anesthesia).

1. Place the thumb and index finger on either side of the neck or at the base of the skull, or alternatively, press a rod at the base of the skull.
2. With the other hand, quickly pull the base of the tail or the hind limbs, causing separation of the cervical vertebra from the skull.
3. Confirm separation by palpation of the cervical region.

ii. Decapitation

Important: Check scissors or other devices used for decapitation annually or more often as needed, depending on the species involved and the frequency of use. A log must be kept that indicates when the equipment was checked and by whom.

1. Use an appropriate size guillotine. Decapitate neonates using scissors or scalpel. Check scissors and scalpels to ensure sharpness and proper function.
2. Maintain the equipment used for decapitation in good working order, and service on a regular basis to ensure sharpness of blades.

Note: The use of plastic cones to restrain animals is recommended because it appears to reduce distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal in the guillotine.

1. Quickly separate the head from the body at the cervical level.

iii. Exsanguination

1. Deeply anesthetize the animal.
2. Verify that withdrawal reflex is absent by pinching the toes.
3. For cardiac puncture, insert a needle (~23 G) at a 30° angle to the left junction formed by the sternal appendix and the last rib.
4. For abdominal aorta puncture, incise the abdomen and retract viscera to expose the aorta. Insert a needle (~23 G for the rat, 25 G for the mouse) into the vessel.
5. Withdraw the maximal volume of blood (~1 mL for the mouse, and ~10 mL for the rat).

Important: Verify that an animal is dead before disposing of the carcass, by making sure there is no respiratory movement for at least 3 minutes.

6. If the animal is not dead (or for additional security), follow the exsanguination by another method of euthanasia (for instance, cervical dislocation).

NOTE: Use of Rodent Carcasses to Feed Raptors and Reptiles

1. Only rodents euthanized by CO₂ or cervical dislocation without anesthetics can be used to feed raptors and reptiles.
2. Genetically modified animals cannot be used as a source of food. Conventional inbred or outbred strains can be used, as well as wild-type progeny.
3. Animals diagnosed with an infectious agent or intentionally infected with an infectious agent cannot be used as a food source.
4. Animals given experimental drugs cannot be used as a source of raptor food.

4.0 Safety

- 4.1 Do not use guillotines unless properly trained.
- 4.2 Have an appropriate scavenging system in place when using inhalant anesthetics for euthanasia.
- 4.3 Properly dispose of blades/needles/syringes in sharps container.

5.0 Contingencies

- 5.1 Contact PI, University Veterinarian or Facility Manager if there is a question on the use or maintenance of a guillotine.
- 5.2 Euthanize neonates less than 10 days old with inhalant anesthetic overdose, barbiturate overdose, or decapitation.