



**Cornell University**  
**Cornell Center for Animal Resources and Education**

**CARE301.01 Rodent Euthanasia**

The intent of this standard operating procedure (SOP) is to describe the different recognized methods for rodent euthanasia. This SOP is intended for personnel trained in euthanizing rodents. This procedure is approved by the Institutional Animal Care and Use Committee (IACUC) and the Cornell Center for Animal Resources and Education (CARE). Any exemption must be approved by the IACUC prior to its application.

**TABLE OF CONTENTS**

1. Introduction
2. Materials
3. Procedures
4. Safety
5. Contingencies
6. References

---

**1. Introduction**

- a. Ensure that all individuals responsible for euthanasia are appropriately qualified and monitored, and adhere to IACUC-approved protocols and institutional policies.
- b. Maintain equipment to ensure optimal performance.

**2. Materials**

- Euthanasia chamber
- CO<sub>2</sub> 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
- Animal restrainer (i.e., Decapicone)
- Sharps container
- Bag or container for animal carcass disposal

**3. Procedures**

a. Non-physical Methods

i. CO<sub>2</sub> asphyxiation

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

1. Place the lid connected to the CO<sub>2</sub> 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.
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 minutes.

4. If the animal is not dead (or for additional security), follow the CO<sub>2</sub> 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 container. If this latter method is used, expose the animal only to the vapor.
2. Vapors are inhaled until respiration ceases 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 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 120 mg/kg of commercial euthanasia solution at recommended dosage 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).

b. Physical methods

i. Considerations

1. Use these techniques only when scientifically justified by the user and approved by the IACUC.
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.

ii. 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.

iii. 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 (see note above).
2. Maintain the equipment used for decapitation in good working order, and service on a regular basis to ensure sharpness of blades. Refer to CARE SOP 309 "Maintenance of Decapitation Equipment".

**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.

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

iv. Exsanguination

1. Deeply anesthetize the animal, as per CARE SOP 101 "Rodent Anesthesia"
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).

v. Use of Rodent Carcasses to Feed Raptors and Reptiles

1. Only rodents euthanized by CO<sub>2</sub> 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. Safety

- a. Do not use guillotines unless properly trained. Refer to CARE SOP 309 "Maintenance of Decapitation Equipment".
- b. Have an appropriate scavenging system in place when using inhalant anesthetics for euthanasia. Refer to CARE SOP 712 "Waste Anesthetic Gas Scavenging Systems".
- c. Properly dispose of blades/needles/syringes in sharps container. Refer to CARE SOP 711 "Sharps Precautions".
- d. For animal related injury refer to CARE SOP 707 "Animal Related Injury".
- e. Refer to Care web pages for information about animal related allergies and zoonosis.

## 5. Contingencies

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

## 6. References

- a. AVMA Guidelines on Euthanasia, June 2007:  
[http://www.avma.org/issues/animal\\_welfare/euthanasia.pdf](http://www.avma.org/issues/animal_welfare/euthanasia.pdf)
- b. PHS "Implementation of the Revised AVMA Guidelines on Euthanasia  
<http://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-005.html>
- c. USDA Care Policy (Euthanasia on page 6)  
[http://www.aphis.usda.gov/animal\\_welfare/downloads/policy/policy3.pdf](http://www.aphis.usda.gov/animal_welfare/downloads/policy/policy3.pdf)
- d. "PHS Policy on Humane Care and Use of Laboratory Animals Clarification Regarding Use of Carbon Dioxide for Euthanasia of Small Laboratory Animals"; Notice: NOT-OD-02-062; National Institutes of Health (NIH), Bethesda, MD. July 17, 2002.  
<http://grants.nih.gov/grants/guide/notice-files/NOT-OD-02-062.html>
- e. CARE SOPs Referenced:
  - CARE SOP. 309 Maintenance of Decapitation Equipment  
<http://www.research.cornell.edu/CARE/documents/SOPs/CARE309.pdf>
  - CARE SOP.101 Rodent Anesthesia  
<http://www.research.cornell.edu/CARE/documents/SOPs/CARE101.pdf>
  - CARE SOP 707 Animal Related Injury  
<http://www.research.cornell.edu/care/documents/SOPs/CARE707.pdf>
  - CARE SOP 711 Sharps Precautions  
<http://www.research.cornell.edu/CARE/documents/SOPs/CARE711.pdf>
  - CARE SOP 712 Waste Anesthetic Gas Scavenging Systems  
<http://www.research.cornell.edu/CARE/documents/SOPs/CARE712.pdf>
  - CARE Zoonosis webpage  
<http://www.research.cornell.edu/care/zoonoses.html>
  - Allergens Prevention:  
<http://www.research.cornell.edu/Care/documents/OHS/AllergyPreventionFactSheet.pdf>