1. PURPOSE

1.1. The purpose of this Animal Care and Use Procedure (ACUP) is to describe the different recognized methods for rodent euthanasia. This ACUP is intended for personnel trained in euthanizing rodents. This ACUP is approved by the Institutional Animal Care and Use Committee (IACUC). Any deviation must be approved by the IACUC prior to its implementation.

2. SCOPE

2.1. This document applies to all rodent users at Cornell University.

3. INTRODUCTION

3.1. The term euthanasia means “good death”. Euthanasia techniques should result in rapid loss of consciousness followed by cardiac or respiratory arrest and the ultimate loss of brain function. In addition, the technique should minimize distress and anxiety experienced by the animal prior to loss of consciousness. This ACUP provides guidelines for the humane euthanasia of rodents using several techniques. Contact the Center for Animal Resources and Education (CARE) at Cornell University by e-mailing care@cornell.edu for more information.

4. MATERIALS AND EQUIPMENT

4.1. Euthanasia Equipment

4.1.1. Euthanasia chamber with CO₂ gas supply.
4.1.2. Barbiturates / euthanasia solution.
4.1.3. Decapitation device (i.e., guillotine) or dedicated scissors.

4.2. Support Supplies

4.2.1. Needles and syringes.
4.2.2. Animal restrainer (e.g., DecapiCone).
4.2.3. Sharps container.
4.2.4. Bag or container for animal carcass disposal.

5. PROCEDURE

5.1. General Requirements

5.1.1. All individuals performing euthanasia must be appropriately qualified. Minimal required training is: Module 1, Module 2, and a hands-on rodent class administered by CARE staff; documentation of training is maintained in the individual’s animal use training file.

5.1.2. Adhere to IACUC-approved protocols and institutional policies.

5.1.3. Maintain equipment to ensure optimal performance.

5.2. Non-Physical Methods

5.2.1. CO₂ administration

NOTE: This method is not approved as a sole means of euthanasia for neonates up to 7 days of age; see section 5.4 Euthanasia of Neonates.

5.2.1.1. Compressed CO₂ gas in cylinders is the only acceptable source of CO₂.

5.2.1.2. Place the animal(s) in an appropriate chamber (i.e. dedicated euthanasia chamber, or rodent cage). 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.

5.2.1.3. Connect the compressed CO₂ gas cylinder to the euthanasia chamber via a hose and dedicated chamber lid, or by passing a hose through a lixit hole or similar opening.

5.2.1.4. Open the tank valve and set the flow so to displace 10-30% of the chamber volume per minute (standard size mouse cage 1.4 L/min or rat cage 4.3 L/min) to induce rapid unconsciousness with minimal distress to the animals.

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

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

5.2.1.6. If the animal is deeply unconscious but respirations have not ceased (or for additional security after respirations have ceased), follow the CO₂ administration with another method of euthanasia (e.g., cervical dislocation).

5.2.2. Overdose of inhalant anesthetic

NOTE: This method is not approved as a sole means of euthanasia for neonates up to 7 days of age; see section 5.4 Euthanasia of Neonates.

5.2.2.1. Expose the animal to a high gas concentration using anesthetic vaporizer in a closed container.
5.2.2.2. Vapors are inhaled until respiration ceases and death ensues.

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

5.2.2.3. If the animal is deeply unconscious but respirations have not ceased (or for additional security after respirations have ceased), follow the anesthesia with another method of euthanasia (e.g., cervical dislocation).

5.2.3. Overdose of injectable barbiturate

5.2.3.1. Inject commercial euthanasia solution as per manufacturer's instructions intravenously (IV) or intraperitoneal (IP).

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

5.2.3.2. If the animal is deeply unconscious but respirations have not ceased (or for additional security after respirations have ceased), follow the injection by another method of euthanasia (e.g., cervical dislocation).

5.3. Physical Methods

5.3.1. Considerations

5.3.1.1. All staff utilizing physical methods of euthanasia must be trained and have demonstrated proficiency to CARE veterinary staff.

5.3.1.2. Apply prior anesthesia or sedation whenever possible.

5.3.2. Cervical dislocation

**NOTE:** This method cannot be used as a sole means of euthanasia in animals weighing more than 200 g.

5.3.2.1. 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.

5.3.2.2. With the other hand, grasp the base of the tail between the thumb and forefinger and pinch firmly. Slightly elevate the hindquarters, no more than 20-30 degrees. Quickly and firmly pull hindquarters by the tail base away from head and neck, simultaneously driving thumbnail down behind the base of the skull with the other hand.

5.3.2.3. Confirm cervical vertebrae separation by palpating the neck.

5.3.3. Decapitation

**NOTE:** This method can only be used if required by the experimental design.
**IMPORTANT:** Check scissors or other devices used for decapitation on a regular basis. A log must be kept that indicates when the equipment was checked and by whom.

5.3.3.1. Use an appropriately sized guillotine. Check guillotine, scissors and scalpels to ensure sharpness and proper function (see note above).

5.3.3.2. Decapitation using scissors or a sharp blade is acceptable for altricial neonates less than 7 days of age. For precocial neonates (e.g., Guinea pigs), follow guidelines for an adult.

5.3.3.3. Maintain the equipment used for decapitation in good working order, and service on a regular basis to ensure sharpness of blades. Refer to ACUP 309 Maintenance of Decapitation Equipment.

**NOTE:** The use of plastic cones (i.e., DecapiCone) 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.

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

5.3.4. Exsanguination

5.3.4.1. Deeply anesthetize the animal, as per ACUP 101 Rodent Anesthesia.
5.3.4.2. Verify that withdrawal reflex is absent by pinching the toes.
5.3.4.3. 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.
5.3.4.4. 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.
5.3.4.5. Withdraw the maximal volume of blood (~1 mL for the mouse, and ~10 mL for the rat).

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

5.3.4.6. If the animal is deeply unconscious but respirations have not ceased (or for additional security after respirations have ceased), follow the exsanguination by another method of euthanasia (e.g., cervical dislocation, pneumothorax).

5.4. Euthanasia of Neonates

5.4.1. Generally, neonatal rodents are more resistant to hypoxia than adults of the same species.
5.4.2. Non-physical methods of euthanasia utilizing inhalant agents (e.g., CO₂, inhalant anesthetics) should be followed-up with a secondary technique (e.g., decapitation) in order to ensure death, as all inhalant agents ultimately induce death via hypoxia.
5.4.3. Acceptable procedures for euthanasia of neonatal mice, rats, and hamsters less than 7 days old:

5.4.3.1. Decapitation, see section 5.3.3.
5.4.3.2. Decapitation, preceded by loss of consciousness via: CO₂ or inhalant anesthetic administration, or hypothermia (see also ACUP 101 Rodent Anesthesia).
5.4.3.3. Overdose of injectable barbiturate IP, see section 5.2.3.

5.4.4. For euthanasia of mice, rats, and hamsters greater than 7 days old follow guidelines for adults.
5.4.5. For euthanasia of precocial rodent neonates (e.g., Guinea pigs) follow guidelines for adults.

NOTE: Near-term fetuses should be confirmed non-viable by monitoring for inactivity prior to disposal or processing (e.g., tissue collection/fixation). For additional security, follow guidelines for euthanasia of neonates.

5.5. Use of Rodent Carcasses to Feed Raptors and Reptiles

5.5.1. Only rodents euthanized by CO₂ or cervical dislocation without anesthetics can be used to feed raptors and reptiles.
5.5.2. 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.
5.5.3. Animals diagnosed with an infectious agent or intentionally infected with an infectious agent cannot be used as a food source.
5.5.4. Animals given experimental drugs cannot be used as a source of food.

6. PERSONNEL SAFETY

6.1. Medical Emergencies: CALL 911.
6.2. When working with animals wear appropriate PPE, observe proper hygiene, and be aware of allergy, zoonosis, and injury risks. Refer to the CARE Occupational Health and Safety webpage for more information.

7. ANIMAL RELATED CONTINGENCIES

7.1. Non-emergency veterinary questions and requests for care, email CARE veterinary staff at care@cornell.edu.
7.2. Emergency veterinary care is available at all times including after working hours and on weekends and holidays by calling the CARE pager (1-800-329-2456).

8. REFERENCES

8.3. PHS Implementation of the Revised AVMA Guidelines on Euthanasia

8.4. USDA Care Policy (Euthanasia on page 3.5)

8.5. 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.

8.6. ACUP 101 Rodent Anesthesia

8.7. ACUP 309 Maintenance of Decapitation Equipment

8.8. CARE Occupational Health and Safety webpage:
http://www.research.cornell.edu/care/OHS.html

9. APPENDIX

9.1. None

10. HISTORY

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