SKIDMORE COLLEGE

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

RODENT EUTHANASIA

1. PURPOSE

1.1. The purpose of this Animal Care and Use 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 SOP 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 Skidmore College.

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 SOP provides guidelines for the humane euthanasia of rodents using several techniques. Contact the IACUC (ext. 5178) if your research requires that alternative euthanasia techniques be utilized.

4. MATERIALS AND EQUIPMENT

4.1. Euthanasia Equipment

4.1.1. Euthanasia chamber with CO_2 gas supply.
4.1.2. Euthanex Lid.
4.1.3. Barbiturates / euthanasia solution.
4.1.4. 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 the CITI Euthanasia Module - Course ID: 1827. Documentation of training is to be 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. CO_2 administration *(to be performed in the Procedure Room hood)*

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 CO_2 gas in cylinders is the only acceptable source of CO_2.

5.2.1.2. Place the animal(s) in a standard mouse cage. Unless there is a compelling reason, euthanasia should be conducted in the home cage. If euthanasia cannot be conducted in the home cage, the cage used for euthanasia should be emptied and cleaned between uses. Do not overcrowd the cage. Each animal must have sufficient room to stand on the floor of the cage with all four feet and have sufficient space to turn around and perform normal postural adjustments.

5.2.1.3. Place the Euthanex lid on the animal cage. Make sure that the lid is seated on the top rim of the cage.

5.2.1.4. Connect the compressed CO_2 gas cylinder via a hose to the quick connect fitting on the Euthanex lid.

5.2.1.5. Open the tank valve and set the flow so to displace 50% of the chamber volume per minute to induce rapid unconsciousness with minimal distress to the animals. To determine the rate of flow, use the following equation where height, width, and length are in inches and 61 is the conversion factor for cubic inches to liters:

$$\frac{\text{height x width x length}}{61} = \text{liters x } 0.50 = \text{flow rate/minute}$$

(Note: There are two holes in the top of the lid. These holes must remain clear to allow for the displacement of air.

5.2.1.6. 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.

5.2.1.7. If the animal is deeply unconscious but respirations have not ceased (or for additional security after respirations have ceased), follow the CO_2 administration by another method of euthanasia (e.g. cervical dislocation or decapitation).

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 or soaked gauze in a closed container. If this latter method is used, expose the animal only to the vapor.

5.2.2.2. 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.

5.2.2.3. If the animal is deeply unconscious but respiration 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 (pentobarbital at least 200 mg/kg) intravenous (IV) or intraperitoneal (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.

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.

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 devises 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.

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.

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.

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 Rodent Anesthesia SOP.

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 an 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 respiration have not ceased (or for additional security after respirations have ceased), follow the exsanguination by another method of euthanasia (e.g. cervical dislocation).

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. CO_2, 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 and rats 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: CO_2 or inhalant anesthetic administration (see also Rodent Anesthesia SOP).

5.4.3.3. Overdose of injectable barbiturate IP, see section 5.2.3.

5.4.4. For euthanasia of mice and rats greater than 7 days old follow guidelines for adults.

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 Skidmore Environmental Health and Safety for Academic Affairs webpage (https://skidmore.edu/ehs/) for more information.

7. ANIMAL RELATED CONTINGENCIES

7.1. Non-emergency veterinary questions and requests for care, email animal care staff at ancare@skidmore.edu.

7.2. Emergency veterinary care is available by calling Greenfield Animal Hospital at 518-893-6228 (Attn. Drs. Frank Akawi or Alhley Serfis).

8. REFERENCES

8.1. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition: https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf

8.2. Artwohl J, Brown P, Corning B, Stein S. 2006. Public Statement: Report of the ACLAM Task Force on Rodent Euthanasia. JAALAS. 45(1): 98-105. http://www.aclam.org/content/files/files/public/active/report_rodent_euth.pdf

8.3. PHS Implementation of the Revised AVMA Guidelines on Euthanasia http://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-005.html

8.4. USDA Care Policy (Euthanasia on page 3.5) www.aphis.usda.gov/animal_welfare/downloads/Animal%20Care%20Policy%20Manual.pdf

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: NOTOD-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

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

8.7. ACUP 101 Rodent Anesthesia http://www.research.cornell.edu/care/documents/ACUPs/ACUP101.pdf

8.8. ACUP 309 Maintenance of Decapitation Equipment http://www.research.cornell.edu/care/documents/ACUPs/ACUP309.pdf

9. APPENDIX

9.1. None