
1. PURPOSE

This Standard Operating Procedure (SOP) describes acceptable procedures for rodent euthanasia. It ensures that animals are euthanized in the most humane way possible, minimizing pain and distress, while ensuring compatibility with research objectives.

2. RESPONSIBILITY

Veterinary care staff, animal care staff, principal investigator (PI) and their research staff.

3. CONSIDERATIONS

All animal euthanasia must be performed by appropriately trained personnel approved on the Animal Use Protocol.

Euthanasia procedures must not be performed in the same room where rodents are housed.

All euthanasia procedures must be continuously monitored by the person(s) performing the procedure, until confirmation of euthanasia is complete. Animals must not be left unattended until the procedure is complete.

4. MATERIALS

- 4.1. Isoflurane/CO₂ euthanasia station (calibrated within the last 12 months) with adequate gas scavenging system or filter
- 4.2. CO₂ euthanasia station
- 4.3. General anesthetic or commercial euthanasia solutions

5. EUTHANASIA OF ADULT RODENTS – CHEMICAL METHODS

- 5.1. CO₂ asphyxiation under isoflurane anesthesia:
 - 5.1.1. It is preferable to anesthetize rodents with isoflurane prior to exposure to CO₂ to minimize pain and distress.
 - 5.1.2. In order to minimize stress animals should be euthanized in their home cage. The maximum cage density must be respected. Never pool animals from different cages.
 - 5.1.3. Neonatal animals (up to 10 days of age) are resistant to the hypoxia induced by high anesthetic gas concentrations and exposure to CO₂, therefore, alternative methods are recommended. Isoflurane/CO₂ may be used for narcosis of neonatal animals provided it is followed by another method of euthanasia (e.g. decapitation using sharp blades). Refer to section 7.
 - 5.1.4. Procedure:
 - 5.1.4.1. Chose an adequately sized induction chamber and connect it to the euthanasia station.
 - 5.1.4.2. Place the animal cage, with filter top removed, in the induction chamber.
 - 5.1.4.3. Open the oxygen tank and set the flowmeter to maximum flow rate.
 - 5.1.4.4. Set the isoflurane vaporizer to 5%.
 - 5.1.4.5. Observe the animals closely. Soon after loss of consciousness close the isoflurane vaporizer and the oxygen tank. While the animals are still unconscious, promptly open the CO₂ tank and set the flowmeter to maximum flow rate.
 - 5.1.4.6. Maintain the CO₂ flow until the animal has stopped breathing. Note that the time required for euthanasia can be several minutes.
 - 5.1.4.7. Close the CO₂ flow meter and the valve on the CO₂ tank.
 - 5.1.4.8. Leave the animals in contact with CO₂ for an additional 2 minutes, minimum.
 - 5.1.4.9. Confirm death as described in section 9 before disposing of the animals.

- 5.2. CO₂ asphyxiation:
 - 5.2.1. CO₂ alone should not be used where other methods are practical for the experiment and the species.
 - 5.2.2. Immersion of animals into chambers pre-filled with carbon dioxide is unacceptable.
 - 5.2.3. In order to minimize stress animals should be euthanized in their home cage. The maximum cage density must be respected. Never pool animals from different cages.
 - 5.2.4. Neonatal animals (up to 10 days of age) are resistant to the effects of CO₂, therefore, alternative methods are recommended. CO₂ may be used for narcosis of neonatal animals provided it is followed by another method of euthanasia (e.g. decapitation using sharp blades). Refer to section 7.
 - 5.2.5. Procedure:
 - 5.2.5.1. Place the appropriate sized lid on the animal cage with grid removed.
 - 5.2.5.2. Connect the regulator hose to lid fitting.
 - 5.2.5.3. Do not pre-charge the chamber.
 - 5.2.5.4. Plug in the heater unit if necessary (e.g. if euthanizing multiple cages)
 - 5.2.5.5. Open the CO₂ tank valve.
 - 5.2.5.6. Set the regulator to the appropriate setting, ensuring a gradual-fill rate of less than 40% and greater than 30% of the chamber volume per minute.
 - 5.2.5.7. After the animals have become unconscious, the flow rate can be increased to minimize the time of death. Please note that the time required for euthanasia can be several minutes.
 - 5.2.5.8. Maintain the CO₂ flow until the animal has stopped breathing.
 - 5.2.5.9. Close the flow meter and the valve on the tank.
 - 5.2.5.10. Leave the animals in contact with CO₂ for an additional 2 minutes, minimum.
 - 5.2.5.11. Confirm death as described in section 9 before disposing of the animals.
- 5.3. Barbiturate or injectable anesthetic overdose:
 - 5.3.1. Pentobarbital: inject at a dose of 120mg/kg intravenously or intraperitoneally.
 - 5.3.2. Other injectable anesthetics: inject three times the anesthetic dose intravenously or intraperitoneally.
 - 5.3.3. Animals should be placed in cages in a quiet area to minimize excitement and trauma until euthanasia is complete.
 - 5.3.4. Confirm death as described in section 9 before disposing of the animals.
- 5.4. Overdose of inhalant anesthetic delivered by precision vaporizer:
 - 5.4.1. The anesthetic should be delivered at a high concentration from a calibrated precision vaporizer of an anesthetic machine connected to an adequate scavenging system or air filter. Inhalant anesthetic agents should be administered under controlled conditions with calibrated equipment.
 - 5.4.2. Anesthetic induction chambers should not be overloaded; respect maximum housing density guidelines. Never pool animals from different cages.
 - 5.4.3. Keep induction chambers clean to minimize odors that might distress animals subsequently euthanized.
 - 5.4.4. Neonatal animals (up to 10 days of age) are resistant to the hypoxia induced by high anesthetic gas concentrations, therefore, alternative methods are recommended. Inhalant anesthetics may be used for narcosis of neonatal animals provided it is followed by another method of euthanasia (e.g. decapitation using sharp blades). Refer to section 7.
 - 5.4.5. Animals may need to be exposed for prolonged time periods to ensure death.
 - 5.4.6. Confirm death as described in section 9 before disposing of the animal

6. EUTHANASIA OF ADULT RODENTS – PHYSICAL METHODS

- 6.1. Anesthesia or sedation is necessary prior to physical methods of euthanasia, unless described in the Animal Use Protocol (AUP) and approved by the Facility Animal Care Committee (FACC).
- 6.2. Physical methods of euthanasia are also an appropriate means to assure death after euthanasia with anesthetics or CO₂ used as euthanasia agents.
- 6.3. Cervical dislocation:
 - 6.3.1. Cervical dislocation, as a primary or secondary method of euthanasia, is not to be used on rats weighing over 200g.
 - 6.3.2. Procedure:
 - 6.3.2.1. Perform the procedure on a flat surface or surface where the animal can grip (e.g., the wire bar grid of the cage).
 - 6.3.2.2. Hold the base of the tail with one hand and allow the animal to stand in a normal position.
 - 6.3.2.3. With the other hand, the thumb and index finger are placed on either side of the neck at the base of the skull. Alternatively, a narrow, blunt instrument such as the dull edge of a scissor blade, acrylic ruler or cage card holder can be used.
 - 6.3.2.4. To accomplish the cervical dislocation, quickly push down and forward with the hand or the object pressed at the base of the skull while pulling backward with the hand holding the base of the tail.

Note: A 2-4 mm space should be palpable at the base of the skull, between the occipital condyles and the first cervical vertebra or within the upper third of the neck.
 - 6.3.3. Cervical dislocation should not be performed as a one-handed technique.
 - 6.3.4. Confirm animal's death by observing the following clinical signs: absence of breathing, pale eyes, no reflexes; animal may urinate.
- 6.4. Decapitation:
 - 6.4.1. Guillotines that are designed to accomplish decapitation in adult rodents in a uniformly instantaneous manner are commercially available.
 - 6.4.2. The use of plastic cones to restrain animals is recommended as it reduces distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal in the guillotine.
 - 6.4.3. Guillotines are not commercially available for neonatal rodents, but sharp blades (e.g. scissors) can be used for this purpose.
 - 6.4.4. Consider using strong and sharp scissors, .e.g., surgical scissors or kitchen shears, for decapitation of adult mice to reduce the risk of injury to personnel.
 - 6.4.5. The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades.
- 6.5. Pneumothorax:
 - 6.5.1. Once death has been confirmed, cut through the skin and muscle of the abdomen just below (caudal to) the thorax.
 - 6.5.2. Lacerate the diaphragm with a sharp pair of scissors or remove the heart to ensure death.
- 6.6. Exsanguination:
 - 6.6.1. Training and skill is required to obtain large enough amounts of blood to ensure true exsanguination. This method is therefore not considered as a true physical method of euthanasia.
 - 6.6.2. Animals may be exsanguinated to obtain blood products, but only when they are deeply anesthetized or immediately following euthanasia using isoflurane and CO₂ or CO₂.
 - 6.6.3. Procedure:
 - 6.6.3.1. Deeply anesthetize animal according to Rodent Anesthesia SOP.
 - 6.6.3.2. Collect blood from the heart.
 - 6.6.3.3. Confirm death as described in sections 6.3, 6.4, or 6.5 before disposing of the animals.

7. EUTHANASIA OF NEONATAL RODENTS

- 7.1. Euthanize rodents under 10 days old by one of the following procedures:
 - 7.1.1. CO₂ asphyxiation under isoflurane anesthesia followed by decapitation.
 - 7.1.2. CO₂ asphyxiation followed by decapitation.
 - 7.1.3. Barbiturate overdose by intraperitoneal injection.
 - 7.1.4. Overdose of inhalant anesthetic followed by decapitation.
 - 7.1.5. Decapitation (using sharp scissors).
- 7.2. Rodents over 10 days old can be euthanized by the same procedures as adult rodents.

8. EUTHANASIA OF GESTATING RODENTS

- 8.1. Gestating rodents with fetuses before two thirds of the gestation period can be euthanized by the same procedures as adult rodents. Euthanasia of the mother or removal of the fetuses should ensure rapid death of each fetus due to loss of blood supply and non-viability of the fetuses at this stage of development.
- 8.2. During the final third of the gestation, fetuses should be given the same ethical considerations as apply to the fully mature animal.
- 8.3. Gestating rodents with fetuses over 12 days, >E12:
 - 8.3.1. CO₂ asphyxiation under isoflurane anesthesia of the mother, followed by decapitation or barbiturate overdose by intraperitoneal injection of the fetuses.
 - 8.3.2. CO₂ asphyxiation of the mother, followed by decapitation or barbiturate overdose by intraperitoneal injection of the fetuses.
 - 8.3.3. Overdose of injectable anesthetics by intraperitoneal injection to the mother, followed by decapitation or barbiturate overdose by intraperitoneal injection of the fetuses.
- 8.4. When the gestational age of the fetuses is unknown, follow procedures detailed in section 8.3.

9. CONFIRMATION OF DEATH

- 9.1. All rodents must be subject to a confirmation of death before disposal of the carcass.
- 9.2. To confirm death, monitor animal for the following signs: no rising and falling of chest, no palpable heart beat, poor mucous membrane color, no response to toe pinch, color change in eyes.
- 9.3. Death can be confirmed using one of the following methods:
 - 9.3.1. Neonates and fetuses over 12 days, >E12:
 - 9.3.1.1. Decapitation
 - 9.3.2. Adult rodents:
 - 9.3.2.1. Physical method as described in section 6.

10. REFERENCES

- 10.1. Carbone L, Carbone ET, Yi EM, Bauer DB, Lindstrom KA, Parker JM, Austin JA, Seo Y, Gandhi AD, Wilkerson JD. "Assessing cervical dislocation as a humane euthanasia method in mice". *J Am Assoc Lab Anim Sci*. 2012 May;51(3):352-6 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3358985/>
- 10.2. American Veterinary Medical Association. [AVMA Guidelines for the Euthanasia of Animals: 2020 Edition](#).
- 10.3. Canadian Council on Animal Care. [CCAC guidelines on euthanasia of animals used in science](#) (2010).
- 10.4. Canadian Council on Animal Care. [CCAC revised guidance on euthanasia using carbon dioxide](#) (July 2020).

SOP REVISION HISTORY

DATE	NEW VERSION
2024.03.15	3. Euthanasia procedures should must not be performed in the same room where rodents are housed.
2024.03.15	5.4.1. The anesthetic can also should be introduced delivered at a high concentration from a calibrated precision vaporizer of an anesthetic machine connected to an adequate scavenging system or air filter. Inhalant anesthetic agents should be administered under controlled conditions with calibrated equipment.
2024.03.15	5.4.2. Anesthetic chambers should not be overloaded and need to be kept clean to minimize odors that might distress animals subsequently euthanized; respect maximum housing density guidelines. Never pool animals from different cages.
2024.03.15	5.4.3. Keep induction chambers clean to minimize odors that might distress animals subsequently euthanized.
2024.03.15	5.4.2. The animal can be placed in a closed receptacle (bell jar) containing cotton or gauze soaked with an appropriate amount of the anesthetic. Because the liquid state of most inhalant anesthetics is irritating, animals should be exposed only to vapors. Avoid direct contact between the animal and the liquid anesthetic. Procedures should be conducted in a chemical fume hood or Type II B2 Biological Safety Cabinet to prevent inhalation of the anesthetic by personnel.
2024.03.15	5.4.3. The anesthetic can also should be introduced delivered at a high concentration from a calibrated precision vaporizer of an anesthetic machine connected to an adequate scavenging system or air filter. Inhalant anesthetic agents should be administered under controlled conditions with calibrated equipment.
2024.03.15	5.4.2. Sufficient air or O₂ must be provided during the induction period to prevent hypoxemia. In the case of small rodents placed in a large container, there will be sufficient O₂ in the chamber to prevent hypoxemia.
2024.03.15	5.4.5. Animals may need to be exposed for prolonged time periods to ensure death.