

STANDARD OPERATING PROCEDURE #301

RODENT EUTHANASIA

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 should 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. Choose 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.

5.3.1. Confirm death as described in section 9 before disposing of the animals.

5.4. Overdose of inhalant anesthetic:

5.4.1. Anesthetic chambers should not be overloaded and need to be kept clean to minimize odors that might distress animals subsequently euthanized.

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.

5.4.3. The anesthetic can also be introduced at a high concentration from a vaporizer of an anesthetic machine connected to an adequate scavenging system or air filter.

5.4.4. Sufficient air or O₂ must be provided during the induction period to prevent hypoxemia. In the case of small rodents placed in a larg (es)-8

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.

SOP REVISION HISTORY

DATE	NEW VERSION
2016.04.24	5.1.4. Animals must not be left unattended until the procedure is complete and all valves, oxygen and CO ₂ , have been closed.
2016.03.16	5.5.4.1. Cervical dislocation, as a primary or secondary method of euthanasia, is not to be used on rats weighing over 200g.
2016.03.16	5.5.5.10. Consider using strong and sharp scissors for decapitation of adult mice to reduce the risk of injury to personnel.
2017.01.27	5.5.5.10. Consider using strong and sharp scissors, surgical scissors or kitchen shears, for decapitation of adult mice to reduce the risk of injury to personnel.
2017.01.27	5.3.1. Inject pentobarbital at a dose of 120mg/kg intravenously or intraperitoneally. 5.3.2. Inject three times the anesthetic dose intravenously or intraperitoneally.
2017.10.23	5.5.7.5. Once death has been confirmed, cut through the skin and muscle of the abdomen just below (caC13.8 (y)1pers thrCC sk.

2020.11.23	<p>2. Responsibility Animal Health Technicians (AHTs) Veterinary care staff, animal care staff, principal investigator (PI) and their research staff.</p>
2020.11.23	<p>3. CONSIDERATIONS All animal euthanasia must be performed by appropriately trained personnel approved on the Animal Use Protocol. Euthanasia procedures should 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.</p>
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