

Name: Rodent Euthanasia
Number: FSU-SOP-600.01
Category: Euthanasia

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1.0 Purpose

This SOP describes the basic procedures for the humane euthanasia of rodents.

2.0 Policy

- 2.1 The practice of euthanasia at FSU will be performed in a manner consistent with the most recent guidelines of the American Veterinary Medical Association (AVMA; see reference 5.1).
- 2.2 All personnel must be trained in accordance with the following procedures prior to performing euthanasia on a rodent, and additionally formally authorized by the PI to perform euthanasia on animals covered by his/her IACUC protocol.
- 2.3 Use of inhalant anesthetics such as isoflurane is only recommended as the preliminary step in the experimental procedure of euthanasia by vascular exsanguination (see SOP 700).

3.0 Materials

- 3.1 CO₂ tank (100% CO₂) with flow meter, pressure regulator, pressure gauge and tubing
- 3.2 Connectors for delivering CO₂ to cage (1-hole stopper plus tubing)
- 3.3 Sharpened scissors
- 3.4 Clear plastic freezer bags for storing carcasses

4.0 Procedure

- 4.1 Euthanasia of adult rodents
 - 4.1.1 Euthanize an animal in its home cage, if possible, to reduce the stress of handling.
 - 4.1.1.1 All animals housed in the same cage (up to 5 adult mice) may be euthanized at the same time by this procedure.
 - 4.1.2 Remove cage (still covered with microisolator top) from ventilated rack in rodent room (101F), spray surface with disinfectant in transfer station, wearing nitrile gloves and lab coat, and remove water bottle from inside cage, before transporting to procedure room (101D) across the hall (on a cart or in a plastic bag).
 - 4.1.3 Place the cage on a cart near the CO₂ tank and connect the hose via connector to the grommet on the back wall of the cage.
 - 4.1.4 Place a non-porous lid over the porous microisolator top to restrict the escape of CO₂.
 - 4.1.5 Turn on the CO₂ at a low initial flow rate @ 10-30% of the volume of the cage per minute.

- 4.1.5.1 This will help to reduce the stress of the procedure, especially by avoiding the irritation produced by introducing the animal into a pre-filled chamber.
- 4.1.5.2 Since ventilated mouse cages are 12x7x8 in (30.5x17.8x20.3 cm; see SOP 200), with a volume of 672 in³ (11.0 L), the initial flow rate should be 1.1-3.3 L/min.
- 4.1.5.3 The flow rate can be increased once the animal stops moving.
- 4.1.6 Leave CO₂ running until the animal has stopped breathing, for a minimum of 2-5 min.
- 4.1.7 Turn off CO₂, and confirm death by the combined signs of ceased respiration, absent toe-pinch withdrawal reflex, and pale eye color.
- 4.1.8 After confirmation of death, perform a secondary method of physical euthanasia to ensure death, such as cervical dislocation, decapitation or pneumothorax induced by bilateral thoracotomy.
- 4.1.9 Properly euthanized animals should be collected in clear plastic freezer bags, with labeling to identify the animal(s) in the bag.
- 4.1.10 If it is necessary to euthanize a large group of animals from different cages, rather than one at a time, as many as 9 adult mice may be transferred collectively in the procedure room from their home cages to a large static cage, and then exposed to CO₂.
 - 4.1.10.1 In this case, the CO₂ hose is attached via connector to a hole on a special microisolator top set aside for this use.
 - 4.1.10.2 The cage is 19x10.5x8 in (48.3x26.7x20.3 cm), with a floor area of 134 in² (864.5 cm²) that can accommodate a maximum of 9 mice @ ≤ 15 in² (96.8 cm²) per mouse (see SOP 200).
 - 4.1.10.3 With volume = 26.2 L (1596 in³), the initial flow rate, @ 10-30% vol/min, should be 2.6-7.9 L/min.
 - 4.1.10.4 These animals must be of the same strain and gender.
 - 4.1.10.5 The cage must be clean, not reused from a previous group euthanasia.
- 4.2 Euthanasia of neonate rodents
 - 4.2.1 Euthanize rodents at postnatal day 10 (P10) or younger by decapitation, using a sharp scissors.
 - 4.2.2 Euthanize older neonate rodents as adults.
- 4.3 Euthanasia of gestating (pregnant) rodents
 - 4.3.1 Euthanize gestating (pregnant) rodents as adults up to E17.
 - 4.3.2 When the pregnancy has extended beyond E17, then the secondary, physical method of euthanasia should be extended to the fetuses.
 - 4.3.2.1 In this case, a bilateral thoracotomy of the dam should be extended into the abdomen to expose the uterine horns and fetuses.
 - 4.3.2.2 Each fetus should then be individually subjected to decapitation with a sharp scissors.

5.0 References

- 5.1 AVMA Guidelines for the Euthanasia of Animals: 2013 Edition.
<https://www.avma.org/KB/Policies/Documents/euthanasia.pdf>
- 5.2 Guidelines for Euthanasia of Rodents Using Carbon Dioxide
<http://oacu.od.nih.gov/ARAC/documents/Rodent Euthanasia Adult.pdf>
- 5.3 Rodent euthanasia (McGill University)
http://www.mcgill.ca/research/files/research/301-rodent_euthanasia_-_may_2017.pdf

SOP REVISION HISTORY

VERSION #	APPROVED	DETAILS
600.01	12/20/17	Authored by T. Schoenfeld