

Guidelines: Euthanasia of Mice and Rats

Objective:	To provide guidance on humanely euthanizing mice and rats
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Note: All euthanasia practices will comply with current editions of the *Guide for Care and Use of Laboratory Animals* and the *AVMA Guidelines for the Euthanasia of Animals* (2020 Edition).

Procedures for Euthanasia by CO₂ or Isoflurane

1. Before euthanasia is performed, steps must be taken to ensure that any live animals within proximate distance of animals being euthanized are protected by a physical barrier sufficient to block visual and olfactory (i.e. pheromonal) indicators of euthanasia, as exposure to these indicators induces substantial distress in the animals nearby. Animal cages within the same euthanasia room should be covered and placed away from the euthanasia chamber. Alternatively, animal cages can be placed in a separate room or area and brought in one cage at a time.
2. Individual or group-housed animals can be placed gently in a clean, uncharged, translucent euthanasia chamber or euthanized in their home cage fitted with a special lid with a special port for delivery of the gas (i.e., CO₂ or isoflurane). **Animals from different cages must not be combined before or during euthanasia, as this causes significant distress.**
3. Animals less than 15 days old must be euthanized separately from adults.
4. When induction chambers are used, animals should be placed in the chamber prior to introduction of CO₂. Gas is to be discharged from a compressed cylinder into the chamber at a flow rate that produces rapid unconsciousness with minimal distress to the animal. The flow rate must be capable of displacing 30% to 70% of the chamber/cage volume per minute. Please refer to **Table 1** below for appropriate flow rates. *Note: Excessive noise or high velocity air movement of CO₂ should be avoided.*

Table 1: Flow Rates for Standard Containers

Container	Flow Rate* (30-70% displacement per minute)
Small mouse cage (10.5" x 6.5" x 6")	2 – 4.7 L/min
Large mouse / rat cage (17" x 8.5" x 10")	7.1 – 16.6 L/min
Euthanasia chamber (177A) (13.5" x 8.5" x 8.5")	4.8 – 11.2 L/min

* **Flow rate calculation:** container volume in liters x 30-70%

5. Each animal must be visually observed during the euthanasia procedure to ensure that animals receive adequate gas concentrations and do not regain consciousness during the terminal procedure. Neonates exposed to CO₂ may take longer to die. Once the neonatal rodent (up to 7d of age) is nonresponsive to painful stimuli, an adjunctive method (decapitation) should be performed.
6. To ensure death, an IACUC-approved secondary method of euthanasia (see **Table 2**) must be employed following CO₂ exposure after cessation of breathing has been observed for at least 1 minute.
7. Carcasses will be bagged and stored in the designated freezer to await removal by Vivarium staff.

8. The euthanizing chamber must be emptied and cleaned in between animals. The chambers and related work area must be thoroughly sanitized after use.
9. Questions should be directed to the Attending Veterinarian.

Table 2: Approved Secondary Methods of Euthanasia

Animal	Age/Weight	Approved Secondary Methods
Mouse or Rat Pups (Neonate)	0-6 days	Decapitation with sharp surgical scissors (requires scientific justification) OR Decapitation following deep CO ₂ or isoflurane anesthesia
Mouse or Rat Pups	7-14 days	Decapitation or cervical dislocation following deep CO ₂ or isoflurane anesthesia
Mouse	15 days - Adult	Cervical dislocation or decapitation following deep CO ₂ or isoflurane anesthesia
Rat	15 days - Adult	Decapitation or thoracotomy following deep CO ₂ or isoflurane anesthesia Cervical dislocation is acceptable for rats under 200g. Personnel must be adequately trained and able to demonstrate proficiency.

Supporting Information

Personnel/Training/Responsibilities

- All personnel performing euthanasia with CO₂ must be trained in this procedure by the Attending Veterinarian or the Attending Veterinarian's designee.
- Hands-on euthanasia training must be repeated at a 3-year interval.

Health & Safety

- All personnel performing euthanasia with CO₂ must be enrolled in the UNCC Occupational Health and Safety program.
- Appropriate laboratory attire must be worn at all times.

Required Materials

- Carbon Dioxide (CO₂) compressed gas cylinder
- Regulator and quick release tubing
- Euthanasia chamber

Guidelines

- All personnel performing euthanasia are expected to review these guidelines and the following references:
 - [AVMA Guidelines for the Euthanasia of Animals](#) (updated 2020; published 01/16/2020).
 - [Guide for the Care and Use of Laboratory Animals](#), Eighth Edition ILAR, NRC, 2011

Quality Control Checks and Acceptance Criteria

- All procedures subject to review by the UNC Charlotte IACUC and Attending Veterinarian.

Revision History

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