



Johns Hopkins University

Animal Care and Use Committee

Euthanasia of Mice and Rats Using Carbon Dioxide²

PURPOSE: This document provides guidance on the correct procedures to follow when euthanizing mice and rats using carbon dioxide.

BACKGROUND: Euthanasia of animals at Johns Hopkins University must be carried out according to the most recent guidance of the American Veterinary Medical Association (AVMA).³ Carbon dioxide (CO₂) inhalation is a common method of euthanasia used for rats and mice. It is the method that will be used by central facilities staff with mice and rats identified for euthanasia by researchers. Use of CO₂ euthanasia by researchers must be included in an ACUC-approved protocol. Appropriate technique, equipment, and source of CO₂ must be used. Compressed CO₂ gas in cylinders is the only approved source because the flow of gas to the euthanasia chamber can be regulated precisely. The practice of immersion, where conscious rodents are placed directly into a container prefilled with 100% CO₂, is unacceptable. **CO₂ generated by other means such as dry ice, fire extinguishers, or chemical means (e.g., antacids) is also unacceptable.**

Upon completion of the procedure, death must be confirmed for each animal by one or more of the methods listed below, as approved in the ACUC protocol. It is important to understand that short-term CO₂ exposure produces anesthesia. So, failure of the animal to move or show a reflex response is not sufficient to confirm death. **Disposal of an anesthetized, rather than a euthanized, animal is a serious animal welfare concern.** Understanding how to avoid this is the responsibility of anyone carrying out euthanasia with CO₂.

PROCEDURES: For euthanasia requests to animal care staff: Do not combine animals from different cages and do not leave pups under 21 days of age in the cage without the dam.

Follow the guidance below when euthanizing animals yourself.

1. Preferably, euthanize animals in the home cage to minimize the stress of being placed into an unfamiliar enclosure and to prevent social aggression. Less ideally, a single adult mouse, 1 litter, or up to 3 weanlings can be euthanized in a critter carrier (small cardboard container). Animals in the euthanasia chamber should be readily visible. All animals in the chamber must be able to make normal postural adjustments.

¹ Approved by the JHU Animal Care and Use Committee on: April 17, 2003---revised 9/28/2006, 7/17/08, 10/15/18, 8/20/2020, 9/17/2020, 1/31/2024.

²AVMA Guidelines on Euthanasia of Animals: 200 Edition. Available at www.jhu.edu/animalcare/

2. If euthanizing selected animals in a social group, transfer the animals to be retained to a new cage; and euthanize the intended animals in the home cage.
3. On occasion, it may be useful to hold more than 5 mice per cage (e.g., mice being collected for immediate euthanasia). This is acceptable but only as long as the following conditions are met:
 - a. Up to 10 compatible mice may be placed in a temporary holding cage for up to 30 minutes, but only if the holding cage is not left unattended.
 - b. If fighting is observed, immediately separate the animals.
 - c. Adult males \geq 6 weeks old from different cages should not be combined.
 - d. If **only** euthanizing pups less than 10 days of age, up to 2 litters may be combined in a single cage.
4. Without prefilling the chamber, place the animals in the chamber and introduce 100% CO₂ at a displacement rate of 30-70%. Humane application of CO₂ requires the use of a pressure-reducing regulator and flow meter that will generate the recommended displacement rates. To ensure the chamber flow rate displaces at least 30% - 70% of the chamber volume per minute, follow "Operation of CO₂ Station" instructions posted near the chamber. Animals should be exposed to the CO₂ for the applicable duration listed in the table below.

Exposure Time Chart

Age of Animal	Time of gas flow	Time of continued exposure	Total of time exposure
10 day to adult mice	2-4 minutes	3-6 minutes	5-10 minutes
10 day to adult rats	2-4 minutes	5-10 minutes	7-14 minutes
Newborn to 10 days old pups: CO₂ exposure only*	2-4 minutes	48-50 minutes	50-54 minutes
Newborn to 10 days old pups: CO₂ exposure immediately followed by secondary method.**	2-4 minutes	5-10 minutes	7-14 minutes

***Neonates are resistant to the hypoxia-induced effects of CO₂. Thus, CO₂ exposure time must be considerably longer if a secondary method, such as decapitation, is not performed.**

****Under the AVMA 2020 guidance, secondary methods, such as decapitation, may only be performed after the neonate is nonresponsive to painful stimuli (e.g., a toe pinch).**

5. After exposure for the appropriate period of time, verify that each animal is dead. Use the method stated in question 16d of your ACUC protocol to confirm death (see below for examples). Any animals with signs of life (e.g., gasping) must be returned to the CO₂ chamber; or, if approved in your protocol, a secondary method of euthanasia (see list below) may be quickly performed.
6. When disposing of the carcasses, place them in a red biohazard bag. Put the bag(s) in a designated carcass refrigerator or freezer.

Methods of confirming death that may be included in the ACUC-approved protocol

1. Use visual and physical examination to verify that the heart has stopped beating and the animal is not breathing; mucous membranes should be pale or white.
2. Observation that the animal fails to recover within 10 minutes after CO₂ exposure ends.

Secondary methods for completing euthanasia that may be included in the ACUC-approved protocol

3. Cervical dislocation
4. Decapitation
5. Immediate harvest of vital organs (i.e., heart, lungs, or brain)
6. Bilateral thoracotomy (pneumothorax)
7. Exsanguination