



Johns Hopkins University

Animal Care and Use Committee

Euthanasia of Rats and Mice Using Carbon Dioxide¹

PURPOSE: This document provides guidance on the correct procedures to follow when euthanizing rodents 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).² 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 rats and mice 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 inflow of gas to the euthanasia chamber can be regulated precisely. To insure the CO₂ flow rate displaces 10% - 30% of the chamber volume per minute a flow meter must be attached to the regulator which is set at 50psi. **CO₂ generated by other means such as dry ice, fire extinguishers, or chemical means (e.g., antacids) is unacceptable.**

Upon completion of the procedure, death must be confirmed for each animal by one or more of the methods listed below. CO₂ is an anesthetic and 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 and a significant breach of the conditions of JHU's approved Public Health Service Animal Welfare Assurance.**

Understanding how to avoid this is incumbent upon anyone carrying out euthanasia with CO₂.

PROCEDURE: Animals in the euthanasia chamber should be readily visible. Do not overcrowd the chamber by combining multiple cages. All animals in the chamber must be able to make normal postural adjustments.

Sudden exposure of conscious animals to CO₂ concentrations of 70% or greater has been shown to be distressful³. Yet death should be induced as quickly as possible. Without pre-charging the chamber, place the animals in the chamber and introduce 100% CO₂ at 10% - 30% of the chamber volume per minute so as to optimize reduction in distress. Since this is critical to the humane application of CO₂, it is necessary to have a pressure-reducing regulator and flow meter that will

¹ Approved by the JHU Animal Care and Use Committee on: April 17, 2003---revised 9/28/2006, 7/17/08, 1/15/15

² AVMA Guidelines on Euthanasia of Animals: 2013 ed. Available at www.jhu.edu/animalcare.

³ Dannenman, P. J., Stein, S., Walshaw, S. O. Humane and practical implications of using carbon dioxide mixed with oxygen for anesthesia or euthanasia of rats. *Laboratory Animal Science*, 1997, 47: 376-385.

generate the recommended displacement rates. Animals should be exposed to the CO₂ for the duration listed in the table below. Because the acceptable flow rate is a range and size of the chamber will affect the exposure time, the table lists a range of times.

Age of Animal	Time gas flow	Time continued exposure	Total time exposure
14 day to adult mice	2 - 4 minutes	3 - 6 minutes	5 - 10 minutes
14 day to adult rats	2 - 4 minutes	5 - 10 minutes	7 - 14 minutes
Newborn to 14 day mouse or rat pups	2 - 4 minutes	5 - 10 minutes	7 - 14 minutes

Neonatal animals (up to 14 days of age) are resistant to the effects of CO₂ and therefore it is recommended that a secondary physical method follow the CO₂ exposure. This may include decapitation using a sharp blade or cervical dislocation.

Death must be ensured for all animals prior to disposal. One of the methods below must be used unless harvest of vital organs will occur right away. All the methods listed below are approved by the Animal Care and Use Committee, and may be used even if they were not included in your approved protocol.

1. Verification that the heart has stopped beating and the animal is not breathing; paleness of mucosal tissue. (This typically is not very practical if many animals are being sacrificed at one time since it involves handling each one.)
2. Observation that the animal fails to recover within 10 minutes after CO₂ exposure ends.
3. Cervical dislocation.
4. Thoracotomy.
5. Decapitation.