

Animal Care & Use Program Policy



CARBON DIOXIDE EUTHANASIA OF RODENTS

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Background:

The Institutional Animal Care and Use Committee (IACUC) has adopted the following policy to: (1) assist the research community by clarifying the specific procedures relating to the use of Carbon Dioxide (CO₂) and (2) promote best practices and ensure that pain and distress are minimized when euthanizing laboratory rodents.

Policy:

Animals should not experience pain, fear, or other significant stress prior to or during euthanasia. Carbon dioxide is a frequently used euthanasia agent for small laboratory animals due to its rapid onset of action, safety, and availability. Any rodent used for research may be euthanized by CO₂ by following the procedures described below. Species that can be euthanized by using CO₂ include mice of the genus *Mus*, rats of the genus *Rattus*, hamsters, gerbils, and other laboratory rodents. Although CO₂ may be considered an acceptable form of euthanasia for other species the IACUC will require specific justification for CO₂ use in non-rodents.

Procedures:

I. ADMINISTERING CO₂

- a. All personnel administering CO₂ to rodents must be properly trained and adhere to animal care and use protocols, policies, and guidelines. Training on the use of the equipment and appropriate methods of euthanasia is available from the Department of Comparative Medicine Central and Waterfront Campuses and Division of Comparative Medicine West Campus Veterinarians.
- b. Compressed gas is the only acceptable source of CO₂ for euthanizing rodents. Dry ice, fire extinguishers and other sources of CO₂ may not be used.
- c. Euthanasia chambers should be constructed of clear material (e.g. Plexiglas) to allow visualization of animals during euthanasia. Chambers must be kept clean to minimize odors that might distress animals.
- d. Euthanasia should occur in a procedure room or laboratory, away from other rodent housing.

- e. Rodents should be euthanized in their home cages and mixing or combining of animals from multiple cages, into a single cage, should be minimized.
- f. Do not pre-fill the euthanasia chamber with CO₂. Start with room air then slowly fill the chamber with CO₂ over several minutes. CO₂ is heavier than room air, thus the chamber may need to be “purged” between groups of cages. An optimal flow rate should displace 10-30% of the chamber volume per minute. Gas flows should be maintained for at least 1 minute after apparent clinical death.
- g. Fetuses also require extended exposure to inhalant anesthetics, including CO₂. When fetuses are not required for study, the method chosen for euthanasia of a pregnant mother should ensure cerebral anoxia to the fetus and minimally disturb the uterine milieu to minimize fetal arousal. A recommended method for euthanasia of the mothers is CO₂ exposure followed by cervical dislocation.
- h. Neonatal rodents are resistant to the hypoxia-inducing effects of CO₂ and require longer exposure times to the agent, alternative methods must be considered (e.g., injection with chemical agents, cervical dislocation, or decapitation).
- i. A confirmatory method of euthanasia must be used and stated in the protocol. Acceptable confirmatory methods, to be performed after carbon dioxide overdose, include exsanguination, decapitation, cervical dislocation, or bilateral thoracotomy.

II. Exemptions

- a. Methods of euthanasia used will be consistent with the recommendations of the AVMA Guidelines on Euthanasia, unless a deviation is justified for scientific reasons in the animal use protocol and approved by the IACUC.

REFERENCES

1. American College of Laboratory Animal Medicine Report of the ACLAM Task Force on Rodent Euthanasia (ACLAM)
2. American Veterinary Medical Association Guidelines for Euthanasia (2007) (AVMA)
3. Guide for the Care and Use of Laboratory Animals (Guide)
4. National Institutes of Health Guidelines for Euthanasia of Rodents Using Carbon Dioxide (NIH)
5. Office of Laboratory Animal Welfare IACUC Guidebook (OLAW)
6. Public Health Service Policy: Clarification Regarding Use of Carbon Dioxide for Euthanasia of Small Laboratory Animals (PHS)
7. Pritchett, K., D. Corrow, J. Stockwell, and A. Smith. 2005. Euthanasia of Neonatal Mice with Carbon Dioxide. *Comparative Medicine* 55(3):275-281.
8. Pritchett-Corning, K. 2009. Euthanasia of Neonatal Rats with Carbon Dioxide. *JAALAS* 48(1):23-27

9. University of Pennsylvania, Office of Regulatory Affairs, IACUC Guideline Humane Intervention and Endpoints for Laboratory Animal Species,
http://www.upenn.edu/regulatoryaffairs/Documents/CARBON_DIOXIDE_EUTHANASIA_OF_RODENTS_GUIDELINE.pdf