

University of Louisiana at Monroe

School of Pharmacy Animal Vivarium

CARBON DIOXIDE EUTHANASIA OF RODENTS

To the greatest extent possible, animals being euthanized should not experience pain, fear, or other significant stress prior to their death. Carbon dioxide (CO₂) is a frequently used euthanasia agent for small laboratory animals due to its rapid onset of action, safety, low cost, and ready availability ([ACLAM](#)). The University of Louisiana at Monroe Institutional Animal Care and Use Committee (IACUC) has adopted the following guideline to: (1) assist the research community by clarifying the specific procedures relating to the use of CO₂ and (2) promote best practices and ensure that pain and distress are minimized in euthanized laboratory rodents. Guidance is derived from the Public Health Service ([PHS](#)), Office of Laboratory Animal Welfare ([OLAW](#)), National Institutes of Health ([NIH](#)), the American Association of Veterinary Medicine ([AVMA](#)), peer institutions, and laboratory animal medicine experts; this guideline concentrates on the following topics regarding the use of CO₂ euthanasia:

- Species
- Administering CO₂
- Confirming death
- Exemptions

SPECIES

Any rodent used for research may be euthanized by CO₂ by following the guidance described below. Examples include mice of the genus *Mus* and rats of the genus *Rattus*.

ADMINISTERING CO₂

1. All personnel administering CO₂ to rodents must be properly trained ([Guide](#)). All Principal Investigators (PI) must assure that their research staff is duly trained and adhere to animal care and use protocols, policies, and guidelines. Training on the use of the equipment and appropriate method of CO₂ euthanasia are available from the Vivarium staff.

“Institutions must ensure that all individuals responsible for administering CO₂ euthanasia are appropriately qualified and monitored, and that they adhere to IACUC approved protocols and institutional policies.” ([PHS](#))

2. 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.

“[Compressed CO₂ in cylinders is the only...recommended source of CO₂ for euthanasia purposes.]” ([PHS](#))
([AVMA](#)) ([OLAW](#))

3. Euthanasia chambers should be constructed of clear material (e.g. Plexiglas) and must be kept clean to minimize odors that might distress animals subsequently euthanized. Gas should be delivered in a predictable and controllable fashion, at a low-flow rate.

“The euthanasia chamber should allow ready visibility of the animals. Do not overcrowd the chamber: all animals in the chamber must be able to make normal postural adjustments.” ([NIH](#))

4. Euthanasia should occur in a procedure room or laboratory, away from other rodent housing.

“Euthanasia should not be performed in the animal room.” ([NIH](#))

5. When possible, rodents should be euthanized in their home cages.

The accepted and common practice is to group animals for euthanasia. The common process of grouping animals immediately prior to euthanasia should provide each individual animal with the ability to make normal postural adjustments. Alternatively, animals should be euthanized in their

home cage whenever possible. (NIH)

6. Do not “pre-fill” the euthanasia chamber with CO₂. Start with room air then slowly fill the chamber with CO₂ over several minutes (OLAW). CO₂ is heavier than room air, thus the chamber may need to be “purged” between groups of cages.

“Because inspiration of high concentrations of CO₂ is both aversive and painful, a recommended procedure is to place animals into a chamber that contains room air and then to gradually introduce CO₂.” (ACLAM)

7. CO₂ first renders the animal anesthetized and then, with adequate exposure time, will result in death by CO₂ narcosis. Animals should be left for additional time within the euthanasia chamber, after spontaneous movements have ceased, with CO₂ continuing to flow.

“Animals should be left in the container until clinical death has been ensured.” (NIH)

8. Combining rats and mice into a single cage is prohibited.

“It is important to also consider that mixing unfamiliar or incompatible animals in the same container may be distressful.” (PHS)

Resistance to hypoxia results in a prolonged time to unconsciousness when CO₂ inhalation is used as a euthanasia agent in neonatal rodents. The duration of exposure to carbon dioxide varies with the age of the neonate compared with adult rodents.

AGE	Mice (Pritchett et al. 2005)	Rats (Pritchett-Corning 2009)
Non-haired pups 0-6 days	60 minutes	40 minutes
Haired pups, eyes closed 7-13 days	20 minutes	20 minutes
Haired pups, eyes open, preweaning 14-20 days	10 minutes	10 minutes
Weanlings and adults 21+ days	5 minutes	5 minutes

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 mother is CO₂ exposure followed by cervical dislocation (ACLAM).

CONFIRMING DEATH

Since the anesthetic effects of CO₂ are reversible, animals that are prematurely removed from the chamber prior to death can recover (OLAW). Furthermore, death should be confirmed by personnel who have been specifically trained to recognize cessation of vital signs in rodents (Guide). Therefore, all animals being euthanized with CO₂ overdose must also receive a confirmatory method of euthanasia to ensure death. These confirmatory methods, to be performed after carbon dioxide overdose, include exsanguination, decapitation, cervical dislocation, bilateral thoracotomy, or at least 50% additional time in the euthanasia chamber filled with 100% CO₂.

Death must be verified after euthanasia and prior to disposal. Unintended recovery must be obviated by the use of appropriate CO₂ concentrations and exposure times or by other means.” (PHS)

Death of the animal must be ensured prior to disposal of the rodent carcass. Failure to confirm death of an euthanized rodent is a significant non-compliance, reportable to the appropriate regulatory and accrediting agencies.

“Unintended recovery of animals after apparent death from CO₂...constitute[s] serious noncompliance with the PHS Policy and... the IACUC, through the Institutional Official, must promptly provide OLAW with a full explanation of the circumstances and actions taken.” (PHS)

EXEMPTIONS

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 writing by the investigator. Any departures from these guidelines will require the PI to provide strong scientific and clinical justifications for the exemption. These justifications must be submitted and will be reviewed by the IACUC.

REFERENCES

American College of Laboratory Animal Medicine Report of the ACLAM Task Force on Rodent Euthanasia ([ACLAM](#))

American Veterinary Medical Association Guidelines for Euthanasia (2007) ([AVMA](#))

Guide for the Care and Use of Laboratory Animals ([Guide](#))

National Institutes of Health Guidelines for Euthanasia of Rodents Using Carbon Dioxide ([NIH](#))

Office of Laboratory Animal Welfare IACUC Guidebook ([OLAW](#))

Public Health Service Policy: Clarification Regarding Use of Carbon Dioxide for Euthanasia of Small Laboratory Animals ([PHS](#))

Pritchett, K., D. Corrow, J. Stockwell, and A. Smith. 2005. Euthanasia of Neonatal Mice with Carbon Dioxide. *Comparative Medicine* 55(3): 275-281.

Pritchett-Corning, K. 2009. Euthanasia of Neonatal Rats with Carbon Dioxide. *JAALAS* 48(1): 23-27.