

**Subject: Carbon Dioxide for Euthanasia**

Source Institutional Animal Care and Use Committee (IACUC)

Effective Date 9/14/2026

Replaces: 12/11/2023

Applies to: Personnel involved in Research or Teaching involving animals.

Reference: Animal Welfare Act; PHS Policy on Humane Care & Use of Laboratory Animals.
Guide for the Care & Use of Laboratory Animals

Introduction

The Institutional Animal Care and Use Committee (IACUC) provides oversight of the institution's animal care and use program, ensuring compliance with federally mandated regulations, ethical standards, and institutional policies governing the humane use of animals in research and teaching at the University of Colorado Denver | Anschutz. The IACUC evaluates and reviews all activities related to the care and use of animals and determines if the proposed activities are in accordance with regulations.

Policy Statement

This policy outlines appropriate methods for utilizing carbon dioxide (CO₂) for the humane euthanasia of adult rats, mice, guinea pigs, gerbils, and hamsters used in biomedical research, in accordance with the Guide for the Care and Use of Laboratory Animals and the AVMA Guidelines for the Euthanasia of Animals.

- The goal is to afford the animals the least stressful circumstances possible at the time of euthanasia.
- The University of Colorado Denver | Anschutz is obligated to report to the NIH any instances of animals that recover from intended euthanasia.
- Compressed gas cylinders are the only CO₂ source deemed acceptable by the AVMA Guidelines for the Euthanasia of Animals. Carbon dioxide generated by other methods, such as dry ice, chemical means, or fire extinguishers, is not acceptable.
- Animals should be placed in an uncharged chamber/cage, which allows the personnel direct observation of animals. The flow rates should be adequate to rapidly achieve a uniform CO₂ concentration and should displace 30-70% of the chamber/cage volume per minute with a CU Denver | Anschutz IACUC recommended displacement rate of 50%.
 - To determine the appropriate flow rate, use the following formula:
 - Flow Rate (Liters/min) = Chamber volume (L) x Displacement rate (%).
 - Measure the length x width x height of the container in centimeters and then divide by 1000 to convert to Liters.
 - Example - 25 cm x 20 cm x 12 cm = 6,000 cm³, which is 6 L. At the recommended 50% displacement rate the flow rate should be 3 L/min.
- After the animals have lost consciousness, the flow rate may be increased to decrease the time necessary to reach death. The AVMA Guidelines does not recommend pre-charging or pre-filling chambers because exposing conscious animals to high levels of CO₂ may cause immediate pain and distress.
- When performing serial euthanasia procedures in the same chamber, remove the lid and leave it open for at least 2 minutes to allow residual CO₂ to dissipate, so the chamber is not pre-charged for the next animal.
- If euthanasia is not conducted in the home cage, the euthanasia chamber should be emptied and cleaned between uses to minimize odors that might distress animals that are subsequently euthanized.



- Gas flow should be maintained for at least one (1) minute after apparent clinical death (last breath), and animals should be left in the chamber for an additional two to three (2-3) minutes after gas flow has been discontinued.
- Neonatal mice and rats (up to 10 days of age) are resistant to hypoxia. Carbon dioxide is suitable for the induction of narcosis but should be followed by another recognized method of euthanasia. Commonly employed techniques are cutting the cervical spine for assured dislocation or decapitation. For more information, see the [Fetal and Neonatal Rodent Euthanasia Policy](#).
- Death must be assured by a second form of euthanasia. The IACUC recommends the following methods to ensure euthanasia following CO₂ asphyxiation:
 - Bilateral thoracotomy
 - Cervical dislocation
 - Decapitation
 - Exsanguination
 - The only exception to the second form of euthanasia is when using the Euthanex Chamber:
 - The Euthanex Chamber has engineering controls to guarantee appropriate hold times for CO₂ exposure to ensure irreversible euthanasia (1).
 - The Euthanex Chambers are placed in areas restricted to OLAR staff.
 - Trained OLAR staff are the only authorized operators of the Chambers and have access to the operator code.
 - Euthanasia using the Euthanex Chambers will be restricted to non-medical emergencies.
- Overcrowding of the euthanasia chamber/cage is not acceptable.
 - Incompatible or unfamiliar animals should not be mixed in the chamber/cage.
 - Placing live animals with recently deceased animals is not acceptable.
 - The caging density should allow for normal postural movements.
 - Only one species is allowed in the chamber/cage during use.
 - Whenever possible, animals should be euthanized in their home cage and/or in a darkened chamber.
 - It is not recommended that euthanasia be performed in the animal room or in the presence of other animals not destined for euthanasia.
- It is imperative that all individuals responsible for administering CO₂ for euthanasia be qualified and trained appropriately on the technique and equipment. Training is required for all individuals working with rodents, and documentation of such training/experience is required to be maintained.
- Any deviation from the policy concerning euthanasia of rodents using CO₂ will be considered and reviewed by the IACUC on a case-by-case basis.

Compliance and Accountability

In accordance with regulatory requirements, failure to comply with this policy will result in a review by the IACUC. Depending on the severity of the violation, you may be required to complete additional training, and your privileges to conduct animal research at CU Denver | Anschutz may be suspended or completely revoked.

If your research privileges are suspended, your funding agency (e.g., NIH) and relevant regulatory agencies (e.g., USDA) may be informed about your violation of federal and/or local policies regarding the humane use of animals. This notification will have a direct impact on your ongoing funding for animal-related research.

Reference: (1) Hickman, DL. Minimal Exposure Times for Irreversible Euthanasia with Carbon Dioxide in Mice and Rats. Journal of the American Association for Laboratory Animal Science. 2022. 61(3) 283-286.