University of Colorado Denver | Anschutz Medical Campus

IACUC
Institutional Animal Care and Use Committee

Subject:	**Carbon Dioxide for Euthanasia**
Source:	Institutional Animal Care and Use Committee (IACUC)
Effective Date:	12/11/2023
Replaces:	02/10/2020
Applies to:	Personnel involved in research or teaching studies involving animals
Reference:	Animal Welfare Act; PHS Policy on Humane Care & Use of Laboratory Animals; Guide for the Care & Use of Laboratory Animals; AVMA Guidelines on Euthanasia.

Introduction

The Institutional Animal Care and Use Committee (IACUC) maintains oversight review for federally mandated rules and regulations with regard to animal research, ethics, misconduct and biomedical research for the University of Colorado Denver | Anschutz Medical Campus (CU Denver | Anschutz)

Policy Statement

This policy is intended to outline appropriate methods for utilizing carbon dioxide (CO_2) for the humane euthanasia of rodents used in biomedical research. $C0_2$ is a recognized agent for the humane euthanasia of adult rats, mice, guinea pigs, gerbils and hamsters. Therefore, its continued use is recommended 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 CU Denver | Anschutz is obligated to report to the NIH any instances of animals that recover from intended euthanasia.
- Compressed gas cylinders are the only CO_2 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 and flow rates should displace 30-70% of the chamber/cage volume per minute with a CU Denver | Anschutz IACUC recommended displacement rate of 50%. After the animals have lost consciousness, the flow rate may be increased to decrease the time necessary to reach death. Pre-charging the euthanasia chamber/cage is not acceptable. Sudden exposure of conscious animals to high levels of CO_2 has been demonstrated to be stressful.
- When performing serial euthanasia procedures in the same chamber, remove the lid and leave open for 2 minutes to allow residual CO_2 to dissipate so the chamber is not pre-charged for the next animal.
- Gas flow should be maintained for at least one (1) minute after apparent clinical death and animals should be left in the chamber for an additional two to three (2-3) minutes after gas flow has been discontinued.
- Death must be assured by a second form of euthanasia unless performed in the Euthanex Chamber. The IACUC recommends the following methods to ensure euthanasia following CO_2 asphyxiation:
    - Bilateral thoracotomy
    - Cervical dislocation
    - Decapitation
    - Exsanguination
    - Exemption to the second form of euthanasia by use of 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.
- Neonatal mice and rats (up to 10 days of age) are resistant to hypoxia. Carbon dioxide is suitable for induction of narcosis but should be followed by another recognized method for euthanasia. Commonly employed techniques are cervical dislocation or decapitation.
- It is imperative that all individuals responsible for administering CO_2 for euthanasia be qualified and trained appropriately on the technique and equipment. Training is required for all individuals working with rodent and documentation of such training/experience is required to be maintained.
- Any deviation from the policy concerning euthanasia of rodents using CO_2 will be considered and reviewed by the IACUC on a case-by-case basis.

(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.

Per regulatory requirements, failure to comply with this policy may result in notification of your funding agency (e.g. NIH) and regulatory agencies (e.g. USDA) that your research has violated federal and/or local policies regarding the humane use of animals. This notification may affect continuous funding of your animal-related research. Further, depending on the violation, you may be required to take additional training and/or your privilege to conduct animal research at CU Denver | Anschutz might be temporarily suspended or even completely revoked.