Purpose

This document outlines the proper procedure for using carbon dioxide (CO_2) to euthanize rodent species, while avoiding or minimizing discomfort, distress, and pain. For a complete discussion of the conditions and techniques available for rodent euthanasia refer to the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition.

Procedures

The following conditions must be met when performing CO_2 euthanasia on rodent species:

- Euthanasia must be conducted by trained personnel.
- Never leave rodents unattended during euthanasia.
- Compressed CO_2 in gas cylinders is the only acceptable source of CO_2 for euthanasia. CO_2 generated by other methods, such as dry ice, fire extinguishers, or chemical means, is unacceptable.
- Euthanasia should be performed in an animal's home cage whenever possible to decrease stress.
- Euthanasia should always be performed singly or in cohorts of live animals (i.e., live animals must not be placed in the chamber with dead animals).
    - Do not consolidate animals with previously demonstrated aggression.
    - Consolidation of female mice and non-weaned pups from different cages is permitted, when necessary.
    - Consolidation of weaned male mice from different cages is permitted, when necessary, at the euthanasia station ONLY.
        - Do NOT consolidate weaned male mice within the animal housing room.
    - Euthanize animals within 10 minutes upon the start of consolidation to reduce animal stress and potential for injury.
    - A maximum of 10 mice is permitted in a standard mouse shoebox cage.
    - A maximum of 25 mice is permitted in a standard rat shoebox cage.
    - Follow standard housing densities when consolidating rats for euthanasia (1 - 8 rats depending on size).
- Euthanasia chambers must be transparent so that all animals may be observed during the process.
- Animals must not be euthanized in animal housing rooms except during special circumstances, such as during quarantine and/or exposure to infectious agents, on a case-by-case basis.
    - Animals being euthanized must be blocked from view of animals in the housing room.
- **Never pre-fill a cage or chamber with CO_2!**

Conducting Euthanasia

1) CO_2 gas may be delivered to animals by:
    a. placing a Euthanex® lid over the home cage after removing the filter and wire tops;
    b. placing a specially adapted cage lid designed by the animal care staff for euthanasia; or
    c. placing the home cage into an approved host cage system.

2) Begin CO_2 delivery to the cage or chamber by turning on the CO_2 cylinder valve and adjusting the flow meter to a flow rate that allows animals to be exposed to a CO_2 flow rate that displaces 30% to 70% (ideally 45%) of the chamber or cage volume per minute.
    a. See Table 1 below for appropriate flow rates for standard cages used at URI.
    b. If euthanasia is conducted in a cage or chamber not listed in Table 1, see Flow Rate Calculation Instructions.
3) Expected time to unconsciousness is usually 2 – 3 minutes. Gas flow may be increased as loss of consciousness is observed. Maintain flow for at least one (1) minute after apparent cessation of breathing.
    a. If animals are not losing consciousness as expected, turn off the flow meter and CO_2 cylinder valve and contact CBRC staff for assistance.
4) Turn off the flow meter and CO_2 cylinder valve when the euthanasia process is complete.

Confirmation of Death

Per IACUC Policy on Euthanasia of Research Animals, animal death must be verified via a secondary confirmatory method as described below. Unintended recovery of animals after apparent death from CO_2 inhalation (e.g., in a necropsy cooler or morgue cooler) is a serious noncompliance issue that could result in loss of privileges to work with animals and funding.

Confirmatory methods appropriate for ***animals older than 14 days of age*** include, but are not limited to:

- Cervical dislocation
    - Not acceptable for rodents >200 grams body weight
- Decapitation
- Bilateral pneumothorax
- Vital tissue harvest (inclusive of heart, lungs, and/or brain)
- Exsanguination with or without perfusion of a histological fixative via the major blood vessels

Confirmatory methods appropriate for ***neonates 14 days of age or younger*** include, but are not limited to:

- Decapitation
    - This is the preferred method
- Bilateral pneumothorax
- Vital tissue harvest (inclusive of heart, lungs, and/or brain)

Cleaning Up

Dispose of carcasses in a carcass bag, double bagging if necessary, and place bags in the refrigerator or freezer.

Chambers and lids must be cleaned and dried between animals or groups of animals to minimize odors that might distress subsequent animals prior to euthanasia.

References

American Veterinary Medical Association (AVMA). (2020). *Guidelines for the Euthanasia of Animals: 2020 Edition*. https://www.avma.org/KB/Policies/Documents/euthanasia.pdf

National Institutes of Health (NIH). (2024). *Guidelines for Euthanasia of Rodents Using Carbon Dioxide*. https://oacu.oir.nih.gov/system/files/media/file/2024-01/b5_euthanasia_of_rodents_using_carbon_dioxide.pdf

Public Health Service. (2015). *Public Health Service Policy on Humane Care and Use of Laboratory Animals*. https://olaw.nih.gov/sites/default/files/phspolicylabanimals.pdf

University of Michigan Animal Care & Use Program. (2023, July 28). *Guidelines for Rodent Euthanasia Procedures for Investigative Personnel.* https://az.research.umich.edu/animalcare/guidelines/guidelines-rodent-euthanasia-procedures-investigative-personnel/

TABLE 1: CO_2 FLOW RATES FOR STANDARD CAGES USED AT URI

Species	Cage Type	Flow Rate (L/min)
Mice	Nexgen (Allentown)	3.0
	Innovive	3.0
	Euthanex "aquarium"	8.0
Rats and other rodents housed in rat cages	Nexgen (Allentown)	8.0
	Innovive	8.0
	Euthanex "aquarium"	8.0

FLOW RATE CALCULATION INSTRUCTIONS

If euthanasia is conducted in a cage or chamber not listed in Table 1, follow these steps to calculate the CO_2 flow rate in L/min to displace 45% of the volume of the chamber per minute.

1) Measure the chamber's length, width, and height in centimeters.
2) Multiply these measurements (length x width x height) to obtain the chamber volume in cm^3 (equivalent to mL).
3) Convert chamber volume to L by dividing the volume by 1000.
4) Multiply the volume of the cage (in L) by 0.45 to determine the ideal flow rate in liters per minute (L/min).