

STANDARD OPERATING PROCEDURES
DIVISION OF COMPARATIVE MEDICINE
UNIVERSITY OF SOUTH FLORIDA

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TITLE: Carbon Dioxide (CO₂) Euthanasia

1. This procedure outlines the proper methods of using carbon dioxide to euthanize animals while avoiding or minimizing discomfort, distress, and pain.

II. RESPONSIBILITY

1. The Veterinarians oversee all aspects of animal health, and are assisted by all

2. Facility Managers ensure implementation of all procedures.
3. Research staff is required to follow these guidelines.

III. PROCEDURES

1. The AVMA Panel on Euthanasia has stated that CO₂ is acceptable **with conditions** for euthanasia of small rodents.
 - a. **Advantages:**
 1. The rapid depressant, analgesic, and anesthetic effects of CO₂ are well established.
 2. Carbon dioxide is readily available in compressed gas cylinders.
 3. Carbon dioxide is inexpensive, nonflammable, and nonexplosive and poses minimal hazard to personnel when used with properly designed equipment.
 4. Carbon dioxide does not result in accumulation of toxic tissue residues in animals from which food is produced.
 - b. **Disadvantages:**
 1. Substantial and conflicting differences in response to CO₂ inhalation exist between and within species, strains, and breeds, making broad generalizations difficult.
 2. Carbon dioxide, whether administered by prefill or gradual displacement methods, can be aversive to some species, and therefore potential exists to cause distress.
 3. Because CO₂ is heavier than air, layering of gas or incomplete filling of a chamber may permit animals to climb or raise their heads above the effective concentrations and avoid exposure. High concentrations may be distressful to some animals.
 4. Immature individuals and some aquatic and burrowing species may have extraordinary tolerance for CO₂.
 5. Reptiles and amphibians may breathe too slowly for the use of CO₂.
 6. Euthanasia by exposure to CO₂ with O₂ supplementation may take longer than euthanasia by other means.

9. If animals are anesthetized with inhaled agents prior to completing the euthanasia process with CO₂, sufficient time should be allowed to prevent rapid recovery during the wash-in of CO₂ and the subsequent wash-out of inhaled agent
2. **Compressed CO₂ in gas cylinders is the only acceptable source of CO₂** for euthanasia. CO₂ generated by other methods such as from dry ice, fire extinguishers, or chemical means (e.g., antacids) is unacceptable.
3. Only animals of the **same species** should be placed into a chamber at any time.
4. Animals are placed into chambers so that they have **sufficient floor space** and are not overcrowded. **Minimum space requirements as listed in the “Guide” must be met for all rodents at all times.** Cages of rodents are not to be combined for the purpose of euthanasia.
5. Euthanasia of more than one animal at a time should always be performed in cohorts of live animals (i.e., live animals must not be placed in the chamber with dead animals).
6. To reduce stress, rodents should be **euthanatized in their home cage whenever possible.** This can be accomplished by delivering CO₂:
 - a. Via a Euthanex® lid over the home cage after removing the filter and wire tops.
 - b. Via the air supply port of an IVC cage.
7. Alternatively, animal(s) are placed in a clean microisolator box with a Euthanex® lid.
NOTE: Sudden exposure to high concentrations of CO₂ may be distressful to some species. Pre-filling the CO₂ chamber is no longer recommended.
8. CO₂ delivery to the microisolator is accomplished by turning the CO₂ cylinder valve and flow meter on so that animal(s) are slowly exposed to increasing levels of CO₂ at the following flow rates which will displace 30-70% of the cage volume/minute:

| Species | Cage Type | Flow Rate L/min |
|---------|----------------|-----------------|
| Mice | Static (small) | 4 |
| | IVC (w/o lid) | 4 |
| | IVC (w/ lid) | 6 |
| Rat | Static | 10 |
| | IVC (w/o lid) | 10 |
| | IVC (w/ lid) | 10 |

9. Gas flow mays 283.2 286.32 Tm [(S)9n 0 11.04f 350.76 244 33

13. **Animals must not be euthanatized in animal housing rooms** except during special circumstances such as during quarantine and/or exposure to infectious agents.

14. Neonates (up until approximately 10 days) are resistant to euthanasia by CO₂ due to their inherent resistance to hypoxia, and may require prolonged exposure time to any type of inhalant. Consequently, **CO₂ alone should not be used alone as a sole means of euthanatizing neonates**. CO₂ may be used to induce narcosis but must be followed with another acceptable method of euthanasia (e.g., decapitation, cervical dislocation, or bilateral thoracotomy) to ensure death. Euthanasia of feti or prenatal mice should be accomplished immediately after removal from the dam. Incomplete neural development in mouse feti less than 14 days of gestation suggests that pain perception at this age is minimal.

Approved:

Date: