Guidelines for Euthanasia of Rodents Using Carbon Dioxide

Introduction: Carbon dioxide (CO_2) inhalation is a common method of euthanasia used at NIH for small rodents (e.g.., mice, rats, guinea pigs, and hamsters). Although CO_2 is generally considered an acceptable euthanasia agent for small rodents when properly administered, its acceptability is predicated on several critical factors described in the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition (AVMA Guidelines).[1,5] The euthanasia method must be appropriate to the research goals, to the species and age of the animal, be approved in the animal study proposal (ASP), and must conform to the most recent AVMA Guidelines unless a scientific justification has been approved by the Institute/Center (IC) Animal Care and Use Committee (ACUC). The use of the term "rodent" in this guideline refers generally to small rodents as noted above. Euthanasia of other rodents (e.g., squirrels, woodchucks, chinchillas, and fossorial species) should follow practices approved by the AVMA Guidelines and the IC ACUC.

Procedure: When using CO_2, death should be induced as painlessly and quickly as possible. As such, there are a few important aspects of this procedure to consider:

1. Animals must be euthanized by trained personnel in a well-ventilated area using appropriate technique, equipment, and agents.

2. Species should not be mixed during euthanasia.

3. It is recommended that animals be euthanized in their home cage. Another accepted and common practice is to group animals for euthanasia. The process of grouping animals immediately prior to euthanasia must provide each individual animal with the ability to make normal postural adjustments.

4. Whenever practical, euthanasia should not be performed in the animal housing room.

5. The euthanasia chamber should allow animals to be readily visible. Do not overcrowd the chamber.

6. Animals undergoing CO_2 euthanasia in manual chamber (i.e., not in a Euthanex or similar automated system) should be continuously observed throughout the entire procedure.

7. Compressed CO_2 gas in cylinders is the only acceptable source of carbon dioxide as it allows the inflow of gas to the induction chamber to be controlled. Dry ice as a source of CO_2 and/or pre-filled chambers are not acceptable. "Either USP Grade A (medical) or Grade B (industrial) carbon dioxide may be considered acceptable as they each provide a minimum purity for carbon dioxide of 99.0%."[2,7]

8. Without pre-charging the chamber, place the animal(s) in the chamber and introduce 100% CO_2 at a fill rate of 30-70% displacement of the chamber volume per minute with CO_2, added to the existing air in the chamber. This is appropriate to achieve a balanced gas mixture to fulfill the objective of rapid unconsciousness with minimal distress to the animals.[3,4]

a. Example: for a 10-liter volume chamber, use a flow rate of 3-7 liter(s) per minute.
b. Use the formula in Attachment 1 to calculate the appropriate flow rate based on chamber/size.

9. Expected time to unconsciousness is usually within 2 to 3 minutes. Observe each rodent for lack of respiration and corneal opacification (faded eye color). Maintain CO_2 flow for a minimum of 1 minute after respiration ceases. If both cessation of breathing and ocular pallor are observed, then remove the rodents from the cage; otherwise continue exposing them to CO_2. Alternatively, after the initial 2 to 3 minutes of active exposure of CO_2, an additional passive exposure time of 3 minutes reliably results in irreversible euthanasia for mice, and an additional 10.5 minutes of passive exposure after 2 to 3 minutes of active exposure reliably results in irreversible euthanasia for rats[8.] If unconsciousness does not occur within 2 to 3 minutes, the chamber fill rate should be checked. The system should also be examined for a defective flow meter, absence of CO_2 supply, and/or leaks. Appropriate CO_2 concentrations and exposure times will prevent unintended recovery.
10. Upon completion of the procedure, death must be confirmed by an appropriate method, such as ascertaining cardiac and respiratory arrest or noting an animal's fixed and dilated pupils. If death cannot be confirmed, narcosis must be followed by a secondary method of euthanasia, such as cervical dislocation, decapitation, or bilateral thoracotomy.

11. If a home cage cannot be used, the CO_2 euthanasia chamber should be cleaned between each use and at the end of the day to remove debris or pheromones expressed during the previous euthanasia session.[1] Alternatively, a new/unused container should be used with each group.

12. For altricial neonatal animals (up to 10 days of age), an adjunctive method should be performed after a neonate is nonresponsive to painful stimuli[3]. Altricial neonatal animals are resistant to the hypoxia-inducing effects of CO_2 and require significantly longer exposure times to the agent compared to adults. Please refer to the AVMA Guidelines for guidance on age, exposure time, and/or acceptable adjunctive methods (e.g., cervical dislocation or decapitation with a sharp scissors).

13. Rodent fetuses are unconscious in utero and hypoxia does not induce a response. If the uterus is undisturbed after the dam is euthanized, it is unnecessary to remove the rodent fetuses. If the uterus is removed from the dam and compromised, each fetus should be euthanized by an adjunctive method (e.g., cervical dislocation or decapitation with a sharp scissors).

14. Precocial young, such as guinea pigs, should be treated as adults.

References:

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3. AVMA may change guidance for CO2 euthanasia in rodents. JAVMA, 2019, 254(1)31.
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Useful Resources:

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Attachment 1

Rodent Euthanasia with Carbon Dioxide: Calculating Flow Rate

The NIH ARAC Guidelines for Euthanasia of Rodents Using Carbon Dioxide states that a CO_2 fill rate of 30-70% of the chamber volume per minute is to be used when euthanizing rodents. The following example illustrates how to calculate the euthanasia chamber volume, and the maximum & minimum CO_2 displacement rates.

Formula:

$$\frac{\text{Euthanasia Chamber Height X Width X Length (inches)}}{\text{61 Cubic Inches/Liter}} = \textbf{Chamber Volume (Liters)}$$

Chamber Volume (Liters) X Displacement Rate = **Flow Rate (Liters/Minute)**

Example:

Step 1 – Calculate Chamber Volume:

$$\frac{\text{8” High X 10.5” Wide X 10” Long}}{\text{61 Cubic Inches/Liter}} = \textbf{13.77 Liters (Chamber Volume)}$$

Step 2 – Calculate 30% Displacement Rate (minimum CO_2 flow rate):

13.77 Liters X 0.3 CO_2 Displacement Rate = **4.13 Liters CO_2 Flow/Minute (Flow Rate)**

Step 3 – Calculate 70% Displacement Rate (maximum CO_2 flow rate):

13.77 Liters X 0.7 CO_2 Displacement Rate = **9.63 Liters CO_2 Flow/Minute (Flow Rate)**