Guidelines for Euthanasia of Rodents Using Carbon Dioxide

Carbon dioxide (CO_2) inhalation is the most common method of euthanasia used at NIH for small animals (i.e., mice, rats, guinea pigs, and hamsters). Although CO_2 is generally considered an acceptable euthanasia agent for small animals when properly administered, its acceptability is predicated on a number of critical factors as described in the AVMA Guidelines for the Euthanasia of Animals. [1,5] The euthanasia method must be appropriate to the research goals, species and age of the animal, approved in the animal study proposal, and must conform to the most recent AVMA Guidelines on Euthanasia[1] unless a scientific justification has been approved by the IC ACUC.

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 using appropriate technique, equipment, and agents.

2. Species should not be mixed during euthanasia.

3. Animals should be euthanized in their home cage whenever possible. Another accepted and common practice is to group animals for euthanasia. Do not overcrowd the chamber. 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 room.

5. The euthanasia chamber should allow animals to be readily visible.

6. Compressed CO_2 gas in cylinders is the only recommended 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. Without pre-charging the chamber, place the animal(s) in the chamber and introduce 100% CO_2. A fill rate of 30-70% of the chamber volume per minute with CO_2, added to the existing air in the chamber 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.

8. Expected time to unconsciousness is usually within 2 to 3 minutes. Observe each rodent for lack of respiration and faded eye color. Maintain CO_2 flow for a minimum of 1 minute after respiration ceases. If both signs are observed, then remove the rodents from the cage; otherwise continue exposing them to CO_2. If unconsciousness has not yet occurred 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.

9. 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. It is important to verify death after CO_2 exposure. If an animal is not dead, CO_2 narcosis must be followed by a secondary method of euthanasia, such as decapitation, bilateral pneumothorax, or cervical dislocation.

10. 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.

11. Altricial neonatal animals (up to 10 days of age) are resistant to the hypoxia-inducing effects of CO_2. Please refer to the AVMA Guidelines for the Euthanasia of Animals (2020) for guidance on age, exposure time, and/or acceptable adjunctive methods.

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

References

1. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. https://www.avma.org/KB/Policies/Documents/euthanasia.pdf
2. OLAW seminar on the Use of Non-Pharmaceutical-Grade Chemicals and Other Substances in Research with Animals, March 1, 2012. http://grants.nih.gov/grants/olaw/120301_seminar_transcript.pdf
3. AVMA may change guidance for CO2 euthanasia in rodents. JAVMA, 2019, 254(1)31.
4. Danneman PJ, Stein S, Walshaw SO. Humane and practical implications of using carbon dioxide mixed with oxygen for anesthesia or euthanasia of rats. Lab Anim Sci 1997, 47:376-385.
5. Neil L, Weary DM. Behavioral responses of the rats to gradual-fill carbon dioxide euthanasia and reduced oxygen concentrations. Applied Animal Behavior Science 100 (2006) 295-308.
6. OLAW FAQ on the use of carbon dioxide as an acceptable euthanasia agent. https://grants.nih.gov/grants/OLAW/faqs.htm#652. December 2016.

Useful Resources:

- Boivin GP, Bottomley MA, Dudley ES, Schimi PA, Wyatt CN, Grobe N. Physiological, behavioral, and histological responses of male C57BL/6N mice to different CO2 chamber replacement rates. JAALAS, 2016, 55(4), 451-61.
- Conlee KM, et al. Carbon dioxide for euthanasia: concerns regarding pain and distress, with special reference to mice and rats. Lab Animals 39:137-161, 2005.
- Djoufack SM, Amparan AA, Grunden B, Boivin GP. Evaluation of carbon dioxide dissipation within a euthanasia chamber. JAALAS, 2014, 53(4), 404-407.
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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)**