

Guidelines for Euthanasia of Rodents Using Carbon Dioxide

CO₂ inhalation is the most common method of euthanasia used at NIH for mice, rats, guinea pigs and hamsters. Although CO₂ 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

Death by CO₂ should be induced as painlessly and quickly as possible. As such, there are a few important aspects of this procedure to consider:

1. Neonatal animals (up to 10 days of age) are resistant to the hypoxia-inducing effects of CO₂. Please refer to guidance provided in the ARAC Guidelines for the Euthanasia of Rodent Fetuses and Neonates.
2. Rodents must be euthanized by trained personnel using appropriate technique, equipment and agents.
3. Species should not be mixed during euthanasia.
4. 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.
5. Whenever practical, euthanasia should not be performed in the animal room.
6. The euthanasia chamber should allow animals to be readily visible.
7. Compressed CO₂ 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 CO₂ 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
8. Without pre-charging the chamber, place the animal(s) in the chamber and introduce 100% carbon dioxide. A fill rate of about 10% to 30% of the chamber volume per minute with carbon dioxide, 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
 - a. Example: for a 10-liter volume chamber, use a flow rate of 1 to 3 liter(s) per minute.
 - b. Use the formula in the table below to calculate the appropriate flow rate for your chamber/size..

9. Expected time to unconsciousness is usually within 2 to 3 minutes.⁴ Observe each rodent for lack of respiration and faded eye color. Maintain CO₂ 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 CO₂. 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 CO₂ supply, and/or leaks. Appropriate CO₂ 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.¹ It is important to verify death after CO₂ exposure. If an animal is not dead, CO₂ narcosis must be followed by a secondary method of euthanasia, such as bilateral pneumothorax or cervical dislocation.⁵
11. If a home cage cannot be used, the CO₂ 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.

References

1. AVMA Guidelines for the Euthanasia of Animals: 2013 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. 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.
4. 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.
5. 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 Review

- Biovin 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.
- Klaunberg BA, O'Malley J, Clark T, Davis JA. Euthanasia of Mouse Fetuses and Neonates. *Contemporary Top Lab Anim Sc* 2004, 43:(5) 29-34.
- McIntyre AR, Drummond RA, Riedel ER, Lipman NS. Automated mouse euthanasia in an individually ventilated caging system: System development and assessment. *JALAS* 2007, 46 (2), 65-73.
- Moody CM, Chua B, Weary DM. The effect of carbon dioxide flow rate on the euthanasia of laboratory mice. *Laboratory Animals*, 2014, 48 (4), 298-304.
- Pritchett-Corning KR. Euthanasia of neonatal rats with carbon dioxide. *JALAS* 2009, 48 (1), 23-27.
- Report of the ACLAM Task Force on Rodent Euthanasia, August 2005.
- Wong D, Makowska IJ, Weary DM. Rat aversion to isoflurane versus carbon dioxide. *Biology letters*, 2013, 9 (1).

Approved - 09/12/01

Revised - 10/09/02, 10/13/04, 10/10/07, 05/12/10, 05/08/13, 1/25/17

Calculating Rodent Euthanasia Chamber Size & Flow Rate

Enter the euthanasia chamber measurements (inches) into the yellow boxes.

Chamber Height		
Chamber Width		
Chamber Length		

CO2 Flow Rate Calculations (L/min) will appear below in blue boxes.

Chamber Volume (Cubic Inches)		
Chamber Volume (Liters)		
10% Displacement Rate (minimum)*		
20% Displacement Rate		
30% Displacement Rate (maximum)*		

[*See NIH ARAC Guidelines for Euthanasia of Rodents Using Carbon Dioxide for additional details.](#)