



**Hunter College Animal Facility**  
**Guidelines for Euthanasia of Rodents Using Carbon Dioxide**

Rodents must be euthanized by trained personnel using appropriate technique, equipment and agents. This is necessary to ensure a humane death that satisfies research requirements. Death should be induced as painlessly and quickly as possible. 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). Euthanasia should not be performed in the animal room. The euthanasia method must be appropriate to the species, approved in the animal study proposal and must conform to the most recent AVMA Guidelines on Euthanasia (2).

CO<sub>2</sub> inhalation is the most common method of euthanasia for mice and rats. These important guidelines should be followed:

1. Neonatal animals (up to 10 days of age) are resistant to the effects of CO<sub>2</sub>, therefore, alternative methods are recommended (2), (5).
2. The euthanasia chamber should allow ready visibility of the animals. Do not overcrowd the chamber: all animals in the chamber must be able to make normal postural adjustments.
3. Compressed CO<sub>2</sub> 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. Without pre-charging the chamber, place the animal(s) in the chamber and introduce 100% carbon dioxide. A displacement rate of 10% to 30% of the chamber volume per minute with carbon dioxide should be appropriate to achieve a balanced gas mixture to fulfill the objective of rapid unconsciousness with minimal distress to the animals (3). (Example: for a 10-liter volume chamber, use a flow rate of 1-3 liter(s) per minute.) Sudden exposure of conscious animals to CO<sub>2</sub> concentrations of 70% or greater has been shown to be distressful (2).
4. Expected time to unconsciousness is usually within 2 minutes (6). Observe each rodent for lack of breathing and faded eye color. If both signs are observed, then remove the rodents from the cage; otherwise continue exposing them to CO<sub>2</sub>. If unconsciousness has not yet occurred within two minutes, the chamber fill rate should be checked. The system should also be examined for a defective flow meter, absence of CO<sub>2</sub> supply, and/or leaks. Appropriate CO<sub>2</sub> concentrations and exposure times will prevent unintended recovery. Again, death must be verified after euthanasia and prior to disposal.
5. The accepted and common practice is to group animals for euthanasia. The process of grouping animals should provide each individual animal with the ability to make normal postural adjustments. Alternatively, animals should be euthanized in their home cage whenever possible. However, when euthanizing successive groups of animals using the same euthanizing chamber, in order to avoid distress, some consideration should be given to evacuating the chamber of residual CO<sub>2</sub>.

## References

1. NIH Guide for Grants and Contracts. 7/17/2002, notice: OD-02-062.
2. AVMA Guidelines for the Euthanasia of Animals: 2013 Edition  
<https://www.avma.org/KB/Policies/Documents/euthanasia.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. Klaunberg BA, O'Malley J, Clark T, Davis JA. Euthanasia of Mouse Fetuses and Neonates. *Contemp Top Lab Anim Sc* 2004, 43:(5) 29-34.
5. Report of the ACLAM Task Force on Rodent Euthanasia, August 2005.
6. Lee Neil, Daniel M. Weary. Behavioral responses of the rats to gradual-fill carbon dioxide euthanasia and reduced oxygen concentrations. *Applied Animal Behavior Science* 100 (2006) 295-308.