

IACUC Guideline

Use of CO₂ for Euthanasia of Rodents

Euthanasia is the act of killing animals by methods that induce rapid unconsciousness and death without causing pain or distress. Euthanasia of laboratory rodents is often achieved by exposure to carbon dioxide in an enclosed container.

In 2002, the Office of Laboratory Animal Welfare issued PHS Policy on Humane Care and Use of Laboratory Animals guidance to Assured institutions clarifying current requirements regarding the use of carbon dioxide (CO₂) as a euthanasia agent for small laboratory animals. In addition the AVMA Guidelines for the Euthanasia of Animals 2020 contains new requirements for the use of CO₂ for euthanasia of small laboratory animals.

The guidelines for the use of CO₂ at Purdue University for the purpose of euthanizing rodents follow the guidance provided by the Office of Laboratory Animal Welfare and the AVMA Guidelines for the Euthanasia of Animals 2020.

- All individuals administering CO₂ euthanasia must be appropriately trained and adhere to IACUC approved protocols and institutional policies.
- Chambers used to administer CO₂ for euthanasia should allow for visualization of the animal(s).
- Chambers should not be crowded and allow for normal postural movements of the rodents. Incompatible rodents or unfamiliar rodents should not be mixed in the chamber. Rodents should remain in the home cage if possible but if use of the home cage is not possible, the chamber must be cleaned between rodents or groups of rodents.
- Pre-filling of the chamber is not acceptable as it has been shown that high concentrations of CO₂ are distressful to some rodents.
- CO₂ should be introduced into the chamber at a rate of 30-70% of the chamber volume per minute to reduce distress to the rodent (s). **A CO₂ flow meter must be used to regulate the flow of CO₂ into the chamber.**
- To determine the rate of flow to obtain a rate of 30-70% per minute replacement of room air by CO₂ use the following equation;
$$\frac{\text{Height} \times \text{width} \times \text{length}}{61} = \text{liters} \times 0.50 = \text{flow rate/minute}$$

Where height, width and length are in inches and 61 is the conversion factor for in³ to liters.
- Death of the rodent(s) must be verified prior to disposal. Unintended recovery must be avoided by the use of appropriate CO₂ concentrations and exposure times or by other means. A secondary method of euthanasia is recommended to ensure death (e.g. thoracotomy, cervical dislocation).
- Unintended recovery of rodents after apparent euthanasia with CO₂ is a documented occurrence. Such an incident constitutes serious noncompliance from the PHS policy and deviation from the Guide for the Care and Use of Laboratory Animals. Guidance regarding prompt reporting of related serious noncompliance is included in a July 17, 2002 notice published in the NIH Guide for Grants and Contracts (OD-02-062) and posted at <http://grants.nih.gov/grants/guide/notice-files/NOT-OD-02-062.html>

- Compressed CO₂ in cylinders is the only AVMA recommended source of CO₂ for euthanasia purposes.
- Neonatal rodents (up to 10 days) are resistant to the effects of CO₂ and up to 50 minutes can be required to achieve death. CO₂ may be used for narcosis of neonatal rodents provided it is followed by another method of euthanasia (e.g. decapitation).

References:

1. NIH Guide for Grants and Contracts. 7/17/2002, notice: OD-02-062.
<http://grants.nih.gov/grants/guide/notice-files/NOT-OD-02-062.html>
2. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition.
<https://www.avma.org/KB/Policies/Pages/Euthanasia-Guidelines.aspx>
3. Conlee KM, Stephens ML, Rowan AN, King LA. Carbon dioxide for euthanasia: concerns regarding pain and distress, with special reference to mice and rats. *Laboratory Animals* 2005, 39: 137-161.
4. Guidelines for the Euthanasia of Rodent Feti and Neonates. NIH Animal Research Advisory Committee, 2013.
https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/rodent_euthanasia_pup.pdf
5. 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.