

IACUC Guideline 02-01: Directions for CO₂ Euthanasia of Rodents

Purpose: The purpose of this document is to provide directions for rodent euthanasia via CO₂ inhalation.

Roles & Applicability.

According to the University of Wyoming IACUC Policies and Procedures Manual (2015); “All those involved with the use of laboratory animals are responsible for ensuring the health and well-being of the animals used in research and education at the University”. It is the responsibility of the research project PI to ensure that all euthanasia techniques are conducted as detailed in their IACUC-approved Protocol, and that all lab personnel are properly trained on how to properly euthanize their study animals (including neonates or atypical conditions). Additionally, it is the responsibility of the PI to ensure that all equipment works correctly and is up-to-date on all service inspections. Finally, it is the responsibility of the PI to determine which version of the Guideline is the most current, as federal and professional society recommendations may result in revisions being made to this guideline to reflect the most current and humane best practices regarding CO₂ euthanasia in rodent research.

Background.

One of the most commonly-used methods of rodent euthanasia is CO₂ inhalation, which rapidly & painlessly induces loss of consciousness (Boivin 2017). While there is very little scientific evidence supporting its prohibition, this method has been heavily scrutinized (Valentim 2015), which highlights the critical importance of the UW animal research community and the IACUC to ensure that any rodent euthanasia involving CO₂ must be done properly, professionally, and humanely.

Procedures.

According to the current UW Policy, protocol non-compliance includes failure to ensure death of animals after euthanasia procedures (i.e., failed euthanasia). At this time, the only approved method for rodent CO₂ euthanasia at UW is detailed in this Guideline - any deviations from this Guideline that lack prior IACUC approval will be considered non-compliance. The acceptability of this technique is predicated on a number of critical factors detailed in the AVMA Guidelines (Underwood 2020).

1. Rodents must be euthanized by trained personnel on the approved IACUC protocol using appropriate technique, equipment, and agents.
2. Species should never be mixed during euthanasia.
3. Whenever practical, euthanasia should not be performed in the animal room and/or within sight/sound/smell of other living rodents. Rodents waiting to be sacrificed should be placed in another room until euthanasia.
4. Animals should be euthanized in their home cage whenever possible. Otherwise, the euthanasia chamber should allow animals to be readily visible.
5. If animals are to be grouped during euthanasia it should not overcrowd the chamber or result in undue stress to the animal(s).
6. Compressed CO₂ gas in cylinders (either USP Grade A or Grade B; minimum purity of 99.0% CO₂) is the only approved source of carbon dioxide, as it allows the inflow of gas to the induction chamber to be controlled. Dry ice and/or pre-filled chambers are not acceptable sources of CO₂.
7. Without pre-charging the chamber, place the animal(s) in the chamber and introduce 100% CO₂ at a fill rate of 30-70% of the chamber volume per minute.
 - For Example- a 10-liter volume chamber would require a flow rate of 3 – 7L per minute.
 - A CO₂ Flow Rate Calculator can be found on the UW IACUC website.

Effective 07/01/2020

Version 01

Approved by:



Dr. R. Scott Seville, IACUC Chair, University of Wyoming



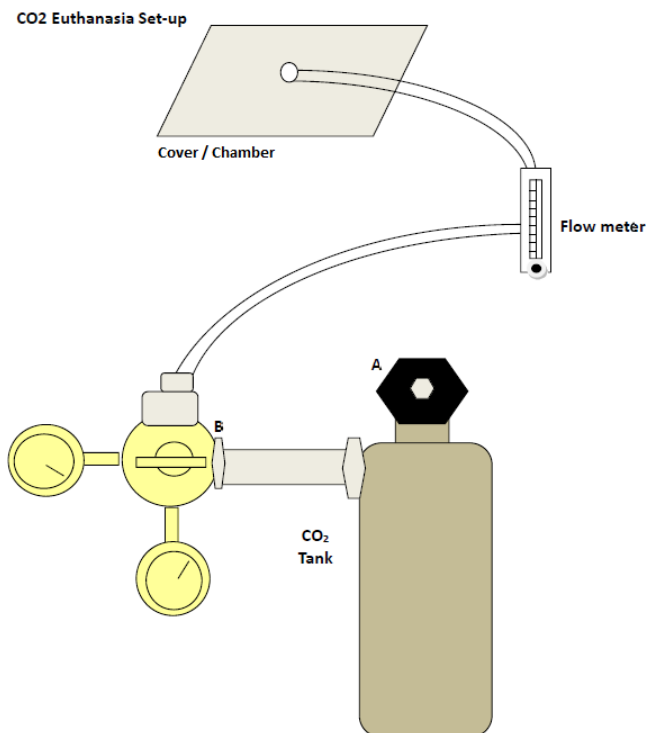
Dr. David R. Evertson, IACUC Attending Veterinarian, University of Wyoming

8. Expected time to unconsciousness is usually 2-3 minutes. Observe until the final animal in the chamber has a complete cessation of respiration and faded eye color. Animals must then remain in the chamber at the current flow rate for an additional 60 seconds, minimum.
9. Upon completion of the procedure it is imperative to confirm the death of each animal. The UW IACUC requires a secondary physical method of euthanasia following CO₂ inhalation and confirmed lack of consciousness. Acceptable methods include: decapitation, cervical dislocation, exsanguination, removal of a vital organ, or the creation of a pneumothorax. The secondary method(s) of choice must be detailed in the IACUC protocol. Cervical dislocation may not be performed in rats > 200 g.
10. If a home cage was not used, the CO₂ euthanasia chamber must be thoroughly cleaned between each use and at the end of the day to remove debris and pheromones.

NEONATES

11. Neonatal animals (up to 10 d) are resistant to CO₂ and can fully recover from 50 minutes of 100% CO₂ exposure (Pritchett 2005). Neonates must be confirmed euthanized via decapitation or similar means detailed in the Protocol Approval Form.

DIAGRAM



A. **CO₂ Tank** – either USP Grade A or Grade B, minimum purity of 99.0% CO₂. Dry ice is NOT an acceptable source of CO₂.

B. **Regulator** – Once turned on, the Flow Meter can be correctly adjusted.

C. **Flow Meter** – Must be set based on cage/chamber size to correctly dispense at a fill rate of 30-70% of the chamber or cage volume/min.

D. **Cage Cover / Euthanasia Chamber** – Should be tight-fitting.

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References.

Boivin, Gregory P., et al. "Review of CO₂ as a euthanasia agent for laboratory rats and mice." *Journal of the American Association for Laboratory Animal Science* 56.5 (2017): 491-499.

Pritchett, K., Corrow, D., Stockwell, J., & Smith, A. (2005). Euthanasia of neonatal mice with carbon dioxide. *Comparative medicine*, 55(3), 275-281.

Underwood, Wendy, et al. "AVMA guidelines for the euthanasia of animals: 2013 edition." Schaumburg, IL: American Veterinary Medical Association, 2020.

Valentim, A. M., et al. "Euthanasia using gaseous agents in laboratory rodents." *Laboratory animals* 50.4 (2016): 241-253.

History of Revisions.

IACUC-G 02-01 – new guideline approved 07/01/2020

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