

University of Tennessee Health Science Center Institutional Animal Care and Use Committee Policy for Euthanasia of Rodents Using Carbon Dioxide

Rodents must be euthanized by trained personnel using appropriate technique, equipment and agents. Death must 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 Whenever practical, euthanasia should not be performed in the animal room. 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

For the purposes of this policy, mice and rats are defined as animals greater than 10 days of age. Neonates are defined as mice and rats from 0-10 days of age. CO₂ inhalation is the most common method of euthanasia for adult mice and rats. A few important aspects of this procedure are:

1. **All personnel who will be performing CO₂ euthanasia must be properly trained.** That training will include face-to-face training by one of the LACU veterinary staff or their designee. That training will be affirmed by each trainee signing a statement they have been properly trained, understand the guidelines, and agree to follow the specified procedure.
2. **When using an automated euthanasia chamber or device, users are expected to follow the posted standard operating procedure.** An SOP that is specific for that automated euthanasia device will be posted immediately adjacent.
3. **Species must not be mixed.** The euthanasia chamber must allow ready visibility of the animals.
4. **Do not overcrowd the chamber or cage used for euthanasia; all animals in the chamber must be able to make normal postural adjustments.**
 - **Requirement**
 - *When animals are euthanized in a standard mouse cage (75 or 78 sq. in) using a Euthanex lid or in a Euthanex chamber, the maximum number of animals per standard mouse cage allowed is 10; for a large mouse (breeder) cage the maximum number of animals allowed is 20.*
5. **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
6. **Without pre-charging the chamber, place the animal(s) in the chamber and introduce 100% carbon dioxide. A fill rate of 10 - 30% of the chamber volume per**

minute with carbon dioxide, added to the existing air in the chamber 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 3 liter(s) per minute.) Sudden exposure of conscious animals to carbon dioxide concentrations of 70% or greater has been shown to be distressful.¹

- **Requirement**

- *All euthanasia chambers provided in LACU are automated to provide a CO2 fill rate consistent with the AVMA recommendations*

7. 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.

- **Requirement**

- *All euthanasia chambers provided in LACU are automated to provide a CO2 fill rate consistent with the AVMA recommendations, maintain flow of CO2 for an appropriate period of time to ensure death, and provide a timer mechanism to signal when the cycle with appropriate wait time has been completed.*
- *When the CO2 euthanasia system cycle has been completed, the operator must verify death of all animals in the chamber and use a secondary method as appropriate and approved in their protocol, properly dispose of the carcasses, take the soiled cage to the properly location, and clean up behind themselves.*
- *CO2 euthanasia stations outside of LACU facilities must have a detailed SOP for operation that is approved by the IACUC.*

8. An accepted and common practice is to group animals for euthanasia. The process of grouping animals immediately prior to euthanasia must provide each individual animal with the ability to make normal postural adjustments. For animals grouped into a standard mouse cage for euthanasia recommendations are listed below. Best practice for animal welfare is that animals should be euthanized in their home cage whenever possible. When euthanizing successive groups of animals using the same cage/container, the euthanizing container should be cleaned between uses to remove the potential distress secondary to remaining pheromones, etc.¹ Alternatively, a new/unused container should be used with each group.

- **Requirement**

- The maximum number of mice occupying a standard mouse cage for the purpose of CO₂ euthanasia is 10
 - The maximum number of mice occupying a large mouse (breeder) cage for the purpose of CO₂ euthanasia is 20
 - For rats, the IACUC approved rat housing density policy must be followed.
9. **Neonatal animals (up to 10 days of age) are resistant to the effects of CO₂ and require prolonged exposure to CO₂.** Alternative procedures including decapitation and anesthetic overdose are recommended for rat and mouse neonates up to 10 days of age.
- **Requirements for neonates:**
 - **Isoflurane overdose using a bell jar. Procedure must be conducted in appropriate ducted biological safety cabinet, fume hood, or with other approved waste anesthetic scavenging device**
 - **Decapitation using sharp scissors.**

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.

Useful Review

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- Pritchett-Corning KR. Euthanasia of neonatal rats with carbon dioxide. *JALAS* 2009, 48 (1), 23-27.
- Wong D, Makowska IJ, Weary DM. Rat aversion to isoflurane versus carbon dioxide. *Biology letters*, 2013, 9 (1).
- 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.

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