

Policies and Procedures

SECTION: IACUC	NUMBER: 7.15			
CHAPTER: Miscellaneous Experimental Animal Use Policies	ISSUED: 11/2007 & 11/08	REV. A: 3/16/09 & 4/7/09	REV. B: 11/26/12 11/25/13	REV. C: 11/25/13 & 3/30/15
POLICY: Euthanasia of Rodents using Carbon Dioxide	REV. D: 08/2016	REV. E: 07/2019	PAGE 1 OF 3	

Purpose

The purpose of this policy is to establish clear guidelines and procedures for rodent euthanasia using carbon dioxide (CO₂) at Creighton University. Performing euthanasia correctly is an ethical imperative. Proper euthanasia is quick, minimizes pain and distress, and reliably causes death. Practical issues such as degree of technical difficulty, time required to perform the procedure and readily available equipment and resources to perform the procedure must be considered, as must aesthetics and human emotion.

Guidelines for humane euthanasia of rodents are provided by the AVMA Guidelines on Euthanasia (American Veterinary Medical Association) and the Report of the ACLAM Task Force on Rodent Euthanasia (Artwohl *et al.* 2006). This policy follows these guidelines and is approved by the Creighton University Institutional Animal Care and Use Committee (IACUC). All investigators using CO₂ as the primary means of euthanasia in rodents will follow this policy unless scientific justification for an exception is provided and approved by the IACUC.

Application(s)

Rodents must be euthanized by qualified personnel using appropriate technique, equipment and agents. This is necessary to ensure a painless death that satisfies research requirements. Death should be induced as painlessly and quickly as possible.

Carbon dioxide inhalation is the most common method of euthanasia for mice, rats, guinea pigs, hamsters and rabbits. The following are minimum standards for CO₂ euthanasia of these species at Creighton.

Preparation:

- “As gas displacement rate is critical to the humane application of CO₂, an appropriate pressure-reducing regulator and flow meter or equivalent equipment with demonstrated capability for generating the recommended displacement rates for the size container being utilized is absolutely

Policies and Procedures

SECTION: IACUC	NUMBER: 7.15			
CHAPTER: Miscellaneous Experimental Animal Use Policies	ISSUED: 11/2007 & 11/08	REV. A: 3/16/09 & 4/7/09	REV. B: 11/26/12 11/25/13	REV. C: 11/25/13 & 3/30/15
POLICY: Euthanasia of Rodents using Carbon Dioxide	REV. D: 08/2016	REV. E: 07/2019	PAGE 2 OF 3	

necessary.” A flow meter calibrated to deliver 20% of the volume of the euthanasia chamber is required in order to comply with the AVMA Guidelines for euthanasia. If you wish to perform CO₂ euthanasia in your laboratory, you must have a flow meter on your CO₂ cylinders.

- **The euthanasia chamber must be clear** to allow complete visibility of the animals.
- Euthanize in home cage if possible. If home cage is not used, the chamber should be emptied and cleaned between uses.
- **Compressed CO₂ gas in cylinders is the only permissible source of CO₂** as it allows the inflow of gas to the induction chamber to be controlled.
- **The chamber must not be pre-charged with CO₂**, as levels above 50% may cause distress in animals and levels above 70% may be painful.
- **All animals must be subjected to a second, physical method of euthanasia/confirmation of death** after apparent death from CO₂. Physical methods of euthanasia include cervical dislocation, decapitation, thoracotomy, or other experimental procedures that ensure death, such as perfusion with fixative, exsanguination, dissection and removal of brain or other major organs. Other means of secondary euthanasia should be proposed only after consultation with the Attending Veterinarian. **The physical method used on a particular animal must be approved on the IACUC protocol covering euthanasia of that animal.**

Procedure:

- **Animals must be monitored throughout the procedure.**
- If recently used, ventilate the chamber with room air to reduce CO₂ levels.
- Do not overcrowd the chamber: all animals in the chamber must be able to make normal postural adjustments. Overcrowded chambers may alter the time in the chamber required for death.

Policies and Procedures

SECTION: IACUC	NUMBER: 7.15			
CHAPTER: Miscellaneous Experimental Animal Use Policies	ISSUED: 11/2007 & 11/08	REV. A: 3/16/09 & 4/7/09	REV. B: 11/26/12 11/25/13	REV. C: 11/25/13 & 3/30/15
POLICY: Euthanasia of Rodents using Carbon Dioxide	REV. D: 08/2016	REV. E: 07/2019	PAGE 3 OF 3	

- **The flow rate for 100% CO₂ should be ~20% of the chamber volume per minute.** (E.g., for a 10-liter volume chamber, use a flow rate of approximately 2 liters per minute.)
- After the animals become unconscious, the flow rate can be increased to minimize the time to death.
- **Animals should be left in the container until all movement, including respiration, has ceased for at least one minute.**
- **Neonatal animals** (up to 10 days of age) are resistant to the effects of CO₂, and therefore the time until apparent death may be extended. Adequate exposure time (up to 50 minutes) should be provided or an adjunctive physical method (e.g. cervical dislocation or decapitation) should be performed after a neonate is unresponsive to painful stimuli.
- **Immediately after removal from the chamber, all animals must be subjected to the approved physical method of euthanasia/confirmation of death.**

References

American Veterinary Medical Association (2013) AVMA Guidelines on Euthanasia. http://www.avma.org/issues/animal_welfare/euthanasia.pdf.

Artwohl, J., P. Brown, B. Corning, and S. Stein (2006) Report of the ACLAM task force on rodent euthanasia. *Journal of the American Association for Laboratory Animal Science* 45:98-105.