Standard Operating Procedure
CO_2 Euthanasia of Rodents

1.0 Scope and Application

This procedure applies to all animal care and research personnel and provides basic instruction for the use of carbon dioxide for euthanasia of rodents.

2.0 Summary of Method

- **Euthanasia of Fetuses**
  - When fetuses are not required for study, euthanasia of the dam should ensure rapid death of the fetuses. Follow the procedures for Euthanasia of Adult Rodents.

- **Euthanasia of Adult and Neonatal Rodents**
  - When possible, animals should be euthanized in the home cage. If euthanasia cannot be conducted in the home cage, chambers should be emptied and cleaned between uses. If animals need to be combined in a single cage they should be of the same species and compatible cohorts. Cage densities must not exceed FSU mouse cage density policies.
  - Ensure flow meter is in the off position before opening the CO_2 valve.
  - Open the valve on the CO_2 cylinder.
  - The cage should be filled at 30% of its volume per minute. Calculate the volume of the cage in liters and multiply this value by 0.3 to determine the flow rate needed:

$$\text{Volume} = \frac{\text{length (cm) x height (cm) x width (cm)}}{1000} = \text{Volume in liters}$$

Volume in liters x 0.3 = flow rate of CO_2 in Liters per Minute (LPM)

  - **If using the cages supplied by Laboratory Animal Resources the flow rates are as follows:**

Cage Type	Flow (LPM)
Mouse Standard Cage	2.0
Mouse Ventilated Cage	2.9
Mouse Large Cage	5.3
Rat Standard Cage	7.5
Vole Standard Cage	2.0

  - Remove microisolator top (if applicable) and place euthanasia lid over cage. Adjust the flowmeter to the calculated flow rate to release CO_2.

- Allow the animals at least 2-3 minutes of CO_2 exposure before removing from the euthanasia chamber.
- Neonatal mice and rats (up to 10 days) are resistant to hypoxia. The duration of exposure to carbon dioxide varies and may require exposures as long as 1 hour to ensure euthanasia.
- Carefully check each animal for heartbeat, respiration or any other signs of life. If the animal is still, continue CO_2 exposure. Recheck each animal to ensure euthanasia.
- For pups less than 10 days of age perform either cervical dislocation or decapitation with sharp scissors on each animal after removal from the euthanasia chamber.
- For all other rodents, cervically dislocate (animals under 200 g only) or perform a thoracotomy on each animal after removal from the euthanasia chamber.
- Carcasses should be placed in a plastic bag labeled with date and the investigator's name. If a necropsy is requested, place the body in the refrigerator. Animal carcasses should be placed in the freezer for disposal purposes.
- Close the valve on the CO_2 cylinder completely.
- Reduce pressure on flow meter and exhaust excess gas by turning the flow meter counterclockwise until the balls falls to zero and then turn off by turning in the opposite direction.
- Commercial euthanasia systems (i.e. Euthanex) must be used according to manufacturer directions. Clear, concise directions for proper use are posted or readily available near these systems.

References

1. American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals: 2020 Edition. Retrieved from https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf.
2. Artwohl J, Brown P, Corning B, Stein S. (August 2005). Report of the ACLAM Task Force on Rodent Euthanasia. Retrieved from http://www.aclam.org/Content/files/files/Public/Active/report_rodent_euth.pdf.
3. 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.
4. National Institute of Health. Animal Research Advisory Council. "Guidelines for Euthanasia of Rodents Using Carbon Dioxide". Web. 14. May. 2013

Revision History
Author: William Hill, DVM, MPH, DACLAM, CPIA
Approval: Date: May 29, 2019
Revision: July 31, 2019
Revision: October 28, 2022, included reference to 2020 AVMA Guidelines only; no additional changes