FSU
ANIMAL CARE & USE COMMITTEE
DIVISION OF RESEARCH

Standard Operating Procedure
Carbon Dioxide (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 (CO_2) for euthanasia of rodents.

2.0 Summary of Method

- Training on the use of the CO_2 equipment (single cage manual system or either of the automated systems) and additional appropriate methods of euthanasia is available from Laboratory Animal Resources (LAR).

- **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, animals should be placed into clean cages or chambers 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.

- **Using the single-cage CO_2 euthanasia station (available in the BRF necropsy, COM necropsy and Psychology 1st floor support area and 3rd and 4th floor necropsy rooms):**
  - Follow instructions posted at each station. These instructions were developed to ensure compliance with the AVMA Guidelines for Euthanasia (2020). Failure to follow the instructions may result in pain or distress to the animal or failure of the euthanasia process.
  - This method requires a second, confirmatory means of euthanasia be applied to animals to ensure death. 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, perform cervical dislocation (animals under 200 g only), exsanguination, bilateral thoracotomy or decapitation on each animal after removal from the cage.
  - Carcasses should be placed in a plastic bag labeled with the date and the investigator's name and placed in a carcass/morgue freezer for disposal purposes.
  - Disposal of an animal that has not been properly euthanized is a serious animal welfare concern and will be considered as non-compliance by the FSU Animal Care and Use Committee.

FSU
ANIMAL CARE & USE COMMITTEE
DIVISION OF RESEARCH

- **Using the multi-cage automated Euthanex SmartBox CO_2 delivery system (available in the BRF necropsy, COM necropsy, King hallway and Psychology 1st floor support area):**
    - The commercial euthanasia system Euthanex SmartBox must be used according to manufacturer directions. Clear, concise directions for proper use are posted or readily available near each of these systems.
    - When using the automated chamber, rodents must be euthanized in their home cages.
    - If stacking cages in the chamber, cages must be slightly offset to allow for CO_2 circulation to the bottom cages. Do not attempt to place more cages in the chamber than will comfortably fit.
    - The automated euthanasia chamber is pre-programmed to ensure that the chamber is slowly filled with CO_2 over several minutes and that the chamber is purged of CO_2 when a cycle ends. Users must select either an Adult or Neonate cycle. Any animals less than 21 days of age must undergo a Neonate cycle. The unit is preprogrammed to ensure that CO_2 is delivered for a sufficient amount of time, including additional time to confirm death.
    - 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 and notify LAR veterinary staff. All other animal carcasses should be placed in the carcass freezer for disposal purposes.

- **Using the single cage automated Euthanex EZ-Dock SmartBox CO_2 delivery system (available in the BRF vivarium near room 177):**
    - This system is only compatible with Techniplast IVC cages.
    - The commercial euthanasia system Euthanex EZ-Dock SmartBox must be used according to manufacturer directions. Clear, concise directions for proper use are posted or readily available at this system.
    - When using this unit, rodents must be euthanized in their home cage.
    - The automated euthanasia chamber is pre-programmed to ensure that the chamber is slowly filled with CO_2 over several minutes. Users must select either an Adult or Neonate cycle. Any animals less than 21 days of age must undergo a Neonate cycle. The unit is preprogrammed to ensure that CO_2 is delivered for a sufficient amount of time, including additional time to confirm death.
    - 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 and notify LAR veterinary staff. All other animal carcasses should be placed in the carcass freezer for disposal purposes.

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
Revision: May 28, 2025