Standard Operating Procedure (SOP)
Rodent Euthanasia using Carbon Dioxide

SCOPE
This SOP applies to Campus Animal Resources (CAR) staff, veterinary staff, and research staff at Michigan State University.

PURPOSE
This SOP describes the procedure for euthanasia of rodents using carbon dioxide (CO\textsubscript{2}) inhalation and overdose. Euthanasia is to be carried out by Campus Animal Resources (CAR) staff and researchers in compliance with the current AVMA “Guidelines for the Euthanasia of Animals” and MSU IACUC recommendations.

DEFINITIONS
Euthanasia: A humane method of ending life that minimizes pain, distress, and anxiety that may be experienced prior to loss of consciousness; causing rapid loss of consciousness followed by cardiac or respiratory arrest and death.

MATERIALS AND EQUIPMENT
Carbon Dioxide (CO\textsubscript{2}) gas cylinder with pressure gauge
Euthanasia Chamber/Cage with euthanasia cover
Flow meter and tubing
Scalpel or scissors

REFERENCE DOCUMENTS
CAR Reference Document 003 (CO\textsubscript{2} Flow Chart)

PROCEDURES
I. General
   A. Euthanize rodents in their home cage whenever possible.
   B. Place only animals of the same species into the chamber.
   C. Do not mix animals from multiple cages into a single cage for euthanasia.
   D. Do not add animals to the chamber once CO\textsubscript{2} administration has started.

   NOTE: Animals must not be euthanized in occupied animal housing rooms except during special circumstances such as during quarantine and/or exposure to infectious agents and only with permission from CAR vet staff.

II. Euthanasia Chamber:
   A. Remove filter/wire tops, and place euthanasia cover lid over the caging unit and connect to the CO\textsubscript{2} cylinder.
      - Alternatively, animal(s) are placed in a clean cage with a cover lid for euthanasia.
   B. Clean chamber lid between animals or groups of animals

   Animals are placed into chambers so that they have sufficient floor space and are not overcrowded. Minimum space requirements as listed in the Guide for the Care and Use of Laboratory Animals must be met for all rodents until time of euthanasia.
III. Gas Delivery and Death Verification:
A. Check to ensure there is an adequate supply of CO2.
B. Review posted signage to follow instructions.
C. Turn on CO2 tank valve by turning the black handle counter-clockwise.

NOTE: Psi level on pressure gauge should be above “0”. Sudden exposure to high concentrations of CO2 may be distressful to the animals. NEVER pre-fill the CO2 chamber with gas prior to placing animals in the chamber. See CAR Reference Document 003 for appropriate flow rates.

D. Deliver CO2 to a cage by turning on the flow meter.
E. Adjust the flow meter based on cage type.
F. Confirm death has occurred – breathing has stopped, absent corneal reflex, no heartbeat can be felt when placing thumb and finger over the rib cage.
G. Turn off CO2 flow meter to 0 L/minute.
H. Turn off CO2 gas cylinder by turning the black handle clockwise.
I. Confirm death by a secondary physical means of euthanasia such as cervical dislocation, exsanguination, decapitation, or bilateral pneumothorax – this confirmation is required prior to carcass disposal.

NOTE: Cervical dislocation in the guinea pig and in rats >200 grams is unacceptable per the AVMA Guidelines.

IV. Carcass Disposal
A. Label bag used for disposal of carcass with your name, PI name, and date.
B. Place carcass in freezer for disposal.

For Euthanasia of neonates:
• **CO2 cannot be used as a sole means of euthanizing neonates less than 10 days of age.** Rodent neonates (up until approximately 10 days) – with the exception of guinea pigs - are resistant to euthanasia by CO2 due to their inherent resistance to hypoxia, and may require prolonged exposure time.
• Euthanasia of pregnant rodents should ensure cerebral anoxia to the fetuses. A recommended method is CO2 exposure of the pregnant rodent, followed by cervical dislocation as a confirmation method.

FIGURE 1: CO2 flow meter and tank pressure gauge