Title: Carbon Dioxide Inhalant Euthanasia
SOP Number: 008
Purpose: The purpose of this SOP is to ensure the humane treatment of animals euthanatized by CO_2.

GENERAL

A. Public Health Service (PHS) Policy (https://olaw.nih.gov/guidance/vertebrate-animal-section.htm) requires Institutional Animal Care and Use Committees (IACUCs) to determine that methods of euthanasia utilized in research proposals are consistent with the Report of the American Veterinary Medical Association Panel on Euthanasia (https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf) (AVMA Panel Report), unless a deviation is justified for scientific reasons in writing by the investigator. IACUC approval of such deviations must be project-specific and include critical review of assertions of scientific necessity. IACUCs may not otherwise disregard or issue blanket waivers of applicable AVMA Panel Report recommendations.
B. Applications and proposals for awards submitted to the PHS must contain, among other things, a description of procedures designed to assure that discomfort and injury to animals will be limited to that which is unavoidable in the conduct of valuable research. Reliance on this overarching principle of minimization of pain and distress is especially useful in resolving apparent inconsistencies and gaps in the scientific literature and the specific guidance on CO_2 use.
C. Although CO_2 is generally considered an acceptable euthanasia agent for small laboratory animals when properly administered, its acceptability is predicated on the following:
    1. High concentrations of CO_2 may be distressful to some species. Accordingly, pre-filling the chamber is recommended only under circumstances in which such use has not been shown to cause distress. While conclusive data are not available for all species, IACUCs and veterinary staff should keep abreast of current peer-reviewed scientific literature and apply informed professional judgment to the design of institutional policies for CO_2 delivery systems and procedures, keeping in mind the imperative to avoid or minimize discomfort, distress, and pain when consistent with sound scientific practices.
    2. Death must be verified after euthanasia and prior to disposal. Unintended recovery must be eliminated by the use of appropriate CO_2 concentrations and exposure times or by other means. (PHS notes that thoracotomy after apparent death from CO_2 is one way to ensure the irreversibility of the procedure).
    3. Institutions must ensure that all individuals responsible for administering CO_2 euthanasia are appropriately qualified and monitored, and that they adhere to IACUC-approved protocols and institutional policies.
    4. Chambers must not be overcrowded. In this regard, it is important to also consider that mixing unfamiliar or incompatible animals in the same container may be distressful.

5. Animals must never be euthanized in the presence of other animals. According to the 2020 AVMA Guidelines for Euthanasia, " Distress vocalizations, fearful behavior, and release of certain odors or pheromones by a frightened animal may cause anxiety and apprehension in other animals.[114,115] Therefore, for sensitive species, it is desirable that other animals not be present when individual animal euthanasia is performed."
6. Compressed CO_2 in cylinders is the only AVMA Panel-recommended source of CO_2 for euthanasia purposes.

POLICY

A. Inhalant euthanasia results in deep depression of all life signs prior to death. This state can be mistaken for death during a cursory examination.
B. To prohibit such an occurrence, the TTU Animal Care and Use Committee has instituted the following policy:
C. Administration of an inhalant overdose must be followed by one of the following procedures:
    1. Cervical Dislocation.
    2. Decapitation.
    3. Exsanguination.
    4. Thoracotomy

PROCEDURE

A. Do not pre-fill the chamber.
B. Place only compatible/socially housed animals in the cage designated for euthanasia Do not exceed 3-5 animals (dependant on species/strain) in a cage to prevent overcrowding and added stress of unfamiliar animals.Do not mix multiple cages for euthanasia.
C. Turn on the CO_2 at a slow rate (oxygen should be displaced at a rate of 30-70%). Flow rate will be dependent on chamber size.
D. For on campus chambers:
    1. Mice: flow rate of 5L/min
    2. Rats: flow rate of 9L/min
E. Maintain the flow rate for at least one minute after respirations have ceased.
F. Turn off the flow of CO_2 . This means turning off the flow meter and the tank or the valve at the wall depending on what unit you are using.
    1. If you are concerned about a CO_2 leak in the centralized system, press the red, emergency air purge button on the wall by the procedure room door, evacuate the room immediately and contact the ACS facilities manager.
G. Remove animal from the chamber and perform approved secondary method of euthanasia as specified in your protocol.

H. If you have more animals to euthanize, turn over the chamber and dump out the CO_2. Then clean the chamber with ACS approved disinfectant or 70% ethanol and repeat the process.