PURPOSE

The purpose of this SOP is to ensure the humane treatment of animals euthanatized by CO₂.

I. GENERAL

Public Health Service (PHS) Policy (http://grants.nih.gov/grants/olaw/references/phspol.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 (http://www.avma.org/resources/euthanasia.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.

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 CO₂ use.

Although CO₂ is generally considered an acceptable euthanasia agent for small laboratory animals when properly administered, its acceptability is predicated on the following:

A. High concentrations of CO₂ 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 CO₂ delivery systems and procedures, keeping in mind the imperative to avoid or minimize discomfort, distress, and pain when consistent with sound scientific practices.

B. Death must be verified after euthanasia and prior to disposal. Unintended recovery must be eliminated by the use of appropriate CO₂ concentrations and exposure times
or by other means. PHS notes that thoracotomy after apparent death from CO₂ is one way to ensure the irreversibility of the procedure.

C. Institutions must ensure that all individuals responsible for administering CO₂ euthanasia are appropriately qualified and monitored, and that they adhere to IACUC-approved protocols and institutional policies.

D. 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. Do not exceed 5 animals in a cage to prevent overcrowding and added stress of unfamiliar animals.

E. Compressed CO₂ in cylinders is the only AVMA Panel-recommended source of CO₂ for euthanasia purposes.

II. 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:

Administration of an inhalant overdose must be followed by one of the following procedures:

2. Decapitation.
3. Exsanguination.
4. Thoracotomy

III. PROCEDURE

A. Do not pre-fill the chamber.
B. Place compatible animals in the cage designated for euthanasia and do not exceed 5 animals at a time in the chamber.
C. Turn on the CO₂ at a slow rate (oxygen should be displaced at a rate of 20%):
   a. 2 L/min for a mouse cage
   b. 4 L/min for a rat cage
D. Maintain the flow rate for at least one minute after the animal has ceased breathing
E. Turn off the flow of CO₂.
F. Remove the animal from the chamber, confirm death either by cervical dislocation, decapitation, exsanguination or thoracotomy.
G. If you have more animals to euthanize: Turn the chamber over to dump out the CO₂ and repeat the process. Clean the chamber to prevent a buildup of waste.
Carbon Dioxide Euthanasia

Although carbon dioxide is generally considered an acceptable form of euthanasia for small laboratory animals when properly administered, its acceptability is predicated on the following:

1. Do not prefill the chamber.

2. Place compatible animals in the cage designated for euthanasia and do not exceed 5 animals in the chamber at a time.

3. Turn on the CO₂ at a slow rate (oxygen should be displaced at a rate of 20%):
   - 2 L/min for a mouse cage
   - 4 L/min for a rat cage

4. Maintain the flow rate for at least 1 minute after the animal has ceased breathing.

5. Turn off the flow of CO₂ and allow it to dissipate before removing animal.

6. Remove the animal from the chamber and confirm that it is dead either by cervical dislocation, decapitation, exsanguination, or thoarcotomy.

7. If you have more animals to euthanize: Turn the chamber over to dump out the CO₂ and repeat the process. Clean the chamber with Clidox to prevent a build-up of waste.