Euthanasia of Laboratory Animals Using Carbon Dioxide (CO₂) Gas

A. Purpose/Scope
This document will describe and outline industry standard methods of euthanasia using CO₂ gas for approved laboratory animal species.

B. Definitions
- Euthanasia – the act of inducing humane death with minimal pain and distress.
- Clinical Death – the state when brain, heart and respiratory function cease.
- Container - The animal's home cage should be used for carbon dioxide euthanasia. If the home cage is not available, then a clean, sturdy, impervious box large enough for the animal to make normal postural adjustments may be used.

C. References
- AVMA Guidelines for the Euthanasia of Animals, 2013 Edition

D. Background
- Euthanasia techniques should result in rapid loss of consciousness followed by cardiac or respiratory arrest and the ultimate loss of brain function.
- Species Covered – rodents
- Mode of action – Direct depression of cerebral cortex, subcortical structures, and vital centers; direct depression of heart muscle. Concentrations of carbon dioxide greater than 30% cause deep anesthesia and death with prolonged exposure.
- Source – Compressed CO₂ gas in cylinders. Carbon dioxide generated by other methods such as from dry ice, fire extinguishers, or chemical means is unacceptable.

E. Procedures
Note: CO2 is not a method of euthanasia for neonates, but can be used as an anesthetic prior to the use of an acceptable method of euthanasia (i.e. decapitation) for neonates.
1. Carbon dioxide gas euthanasia
   a. Only animals of the same species may be euthanized in the same cage.
   b. Animals must not be overcrowded and must be able to make normal postural adjustments.
   c. Within DCM facilities leave the animal in its home cage or clean cage and place the lid affixed to the carbon dioxide hose over the cage. Gradually fill the cage with carbon dioxide by using a flow rate that equates to 10-30% of cage volume/minute. Allow the carbon dioxide gas to flow into the cage for at least one minute after the animal has stopped breathing.
   d. Perform a secondary physical method to ensure death:
      • Cervical dislocation is recommended for mice.
      • Opening the chest with scissors (thoracotomy) is recommended for rats.
      • Cervical dislocation is not acceptable for rats over 200g.
   e. Place the dead animal into a plastic bag and transport it to the carcass freezer.
   f. Within IACUC approved investigators laboratories euthanasia using carbon dioxide is to be performed using an appropriate container. Place animals in the container and using a regulator, adjust carbon dioxide flow rate to a 10-30% of cage volume/minute gradual
displacement of room air. Allow the gas to flow for at least one minute after the animal has stopped breathing.

g. Perform a secondary physical method to ensure death:
   • Cervical dislocation is recommended for mice.
   • Opening the chest with scissors (thoracotomy) is recommended for rats.
   • Cervical dislocation is not acceptable for rats over 200g.

h. Place the dead animal into a plastic bag and transport it to the DCM carcass freezer.

Approved by the Institutional Animal Care & Use Committee

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