CARBON DIOXIDE (CO₂) EUTHANASIA IN MICE AND RATS

Background: According to the American Veterinary Medical Association's Panel on Euthanasia, carbon dioxide, when used properly, is an acceptable form of euthanasia for appropriate species, including mice and rats. Carbon dioxide has many advantages such as rapid depressant, analgesic, and anesthetic effects, ease of availability, and it poses minimal hazard to personnel. Further, it has been shown that CO2 does not distort murine cholinergic markers or corticosterone concentrations. Compressed CO2 gas in cylinders is the only recommended source of carbon dioxide because the inflow to the chamber can be regulated precisely. *Carbon dioxide generated by chemical means (antacids) or by dry ice is unacceptable.*

Policy: Do not overcrowd the cages of mice and rats destined for euthanasia and the CO₂ chamber itself. Each animal must have the minimum amount of floor space consistent with OHSU policy and The Guide for the Care and Use of Laboratory Animals. In general, only 5 adult mice and two adult rats are allowed in their respective shoebox cages. It is recommended that, whenever possible, animals from different cages are **not** mixed and animals should remain in their home cages for transport. If cages are combined, the animals should be compatible. Males from different cages should not be placed in the same cage. If fighting occurs, the animals should be separated immediately.

- Only one species at a time will be placed in the chamber, and animals will be euthanized as cohorts (live animals will never be placed in the chamber or cage with dead animals).
- Animals should first be placed into the chamber followed by administration of a low flow of CO₂. Use of dry ice is unacceptable.
- The gas should displace at least 20% of the chamber volume per minute. It has been shown that prefilling the chamber or applying an immediate high gas flow rate can cause excitability and distress. Gas flow should be maintained for at least 1 minute after apparent clinical death (total gas exposure time can range from 5 to 10 minutes).
- DO NOT LEAVE THE CO₂ CHAMBER UNATTENDED DURING EUTHANASIA.
- Death can be confirmed by absence of respirations and lack of a palpable heartbeat.
- To ensure death, a secondary method of euthanasia should be performed. Secondary methods include cervical dislocation (approved for mice and in rats weighing less than 200g), thoracotomy, exsanguination, or anesthetic overdoses (sodium pentobarbital, mouse/rat cocktail). Alternatively, the plastic bag containing the euthanized animals can be filled with CO₂ prior to disposal.
- After use, the chamber should be cleaned to minimize odors that may induce stress to subsequent animals placed in the chamber.

Neonates: Neonates up to 14 days of age are resistant to the effects of CO₂, therefore, supplemental methods of euthanasia are required. Carbon dioxide can be used to first induce narcosis and then this must be followed with cervical dislocation or decapitation.