

**University of Cincinnati
Animal Care and Use Program**

Euthanasia of Rodents with Carbon Dioxide

In accordance with the AVMA Guidelines on Euthanasia of Animals, the following requirements must be met when rodents are euthanized with carbon dioxide gas (CO₂).

Population Density in Euthanasia Chambers-When animals cannot be euthanized in their home cages or as stable social groups, population density must meet all of the following criteria:

- When combining animals, 20% of the floor space is to be visible.
- The minimization of social stress or harm caused by mixing unknown conspecifics should be avoided (i.e. breeding males, disproportionate animal size, etc.).
- Animals should be combined immediately prior to euthanasia.

CO₂ Source

Carbon dioxide must come from compressed gas and be medical- or technical-grade. The use of dry ice or industrial-grade gas is prohibited.

Flow Rate

The Euthanasia system must provide an optimal flow rate for CO₂ which will displace 30% to 70% of the chamber or cage volume/min (a flow meter and flow regulator may be required for non-commercial systems).

Chambers should not be pre-charged with CO₂ and should be emptied and cleaned between uses.

Ensuring non-recovery

A physical method must be performed to ensure non-recovery after euthanasia with CO₂. This procedure is described in the approved IACUC protocol and must be adhered to for each animal euthanized.

Carcass Storage and Disposal

- Carcasses should be stored in a designated refrigerator or cooler to ensure appropriate disposal.