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**
The IACUC defines the maximum number of rodents permitted in a cage for euthanasia as twice the number of rodents allowed to be housed in a particular cage. For example, no more than 4 adult mice may be placed in a standard shoebox cage for housing; therefore, no more than 8 adult mice may be placed together in a standard shoebox cage for euthanasia.

**CO₂ Source**
Carbon dioxide must come from compressed gas. The use of dry ice is prohibited.

**CO₂ Grade**
Medical-grade or technical-grade carbon dioxide must be used. The use of industrial-grade gas is prohibited.

**Flow Rate**
Euthanasia system must provide an optimal flow rate for CO₂ which will displace 10% to 30% 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.

- For LAMS maintained chamber, flow meters are preset by LAMS to provide the proper flow rate. Do not increase the flow rate. The home cage is recommended but if euthanasia cannot be conducted in the home cage, chambers should be emptied and cleaned between uses.

- For PI maintained euthanasia chambers, the PI is responsible ensuring that at the flow meter is calibrated for the chamber size in use.

**Ensuring non-recovery**
A physical method to ensure non-recovery must be performed after euthanasia with CO₂. This procedure is described in the approved IACUC protocol and must be adhered to for each animal euthanized. Carcasses must be disposed of properly in a timely manner.