

UNIVERSITY OF DELAWARE
OFFICE OF LABORATORY ANIMAL MEDICINE

Use of Carbon Dioxide (CO₂) for Rodent Euthanasia SOP #PRO-012

Objective:

To document the procedures for euthanizing animals using carbon dioxide. Euthanasia is the act of inducing painless and rapid death.

Introduction:

CO₂ inhalation is commonly used for euthanasia procedures. CO₂ has rapid depressant and anesthetic properties. It is reasonably safe for the handler and is an inexpensive method of euthanasia especially for rodents. Compressed gas CO₂ must be used. Dry ice is not acceptable. CO₂ is colorless, odorless and heavier than air.

Safety Considerations:

- Do not use CO₂ tank before receiving instructions from OLAM staff
- Exercise caution when using CO₂ tank as gas is under pressure.
- Do not leave the room while CO₂ tank is in use.

Procedure:

1. Rodents may be euthanized in their cage (preferable) or in a euthanasia chamber. Euthanasia chambers must be cleaned with warm soapy water between groups of animals.
2. Attach a clear hose from the carbon dioxide tank to a lid designed for euthanasia which will be placed on a rat or mouse cage, or to the lid of one of the euthanasia chambers.
3. **DO NOT** pre-charge the chamber with carbon dioxide.
4. Animals should not be overcrowded.
5. Turn the valve on the flow meter to the appropriate level so that the carbon dioxide fills 30 – 70% of the chamber volume/minute (see signs posted for correct flow meter rates for that particular chamber). Flow meter rates using a lid for a mouse cage are 1.9 to 4.6 liters per minute, and for a rat cage are 7.2 to 12 liters per minute.
6. Once animals are unconscious, the flow can be increased.
7. Animals should be exposed until complete cessation of breathing plus at least one minute.
8. Death **MUST** be confirmed according to the approved IACUC protocol.
9. Place the carcasses in carcass bags.
10. Label the bags with investigators name, room number and the date prior to placing the bags into the freezers.

NOTE: Neonatal animals (up to 10 days of age) are resistant to the effects of CO₂. Therefore, alternative methods are required for euthanasia, e.g. decapitation.