BACKGROUND

This procedure outlines the proper methods of using carbon dioxide to euthanize rodent species, while avoiding or minimizing discomfort, distress and pain. For a complete discussion of the conditions and techniques available for rodent euthanasia refer to the AVMA Guidelines for the Euthanasia of Animals: The 2016 Edition. https://www.avma.org/KB/.../AnimalWelfare/.../Humane-Slaughter-Guidelines.pdf

PROCEDURES

The following conditions must be met when performing CO₂ euthanasia:

- Compressed CO₂ in gas cylinders is the only acceptable source of CO₂ for euthanasia. CO₂ generated by other methods such as from dry ice, fire extinguishers, or chemical means is unacceptable.

Animals are placed into chambers so that they have sufficient floor space and are not overcrowded. Minimum space requirements as listed in the, Eighth Edition (https://grants.nih.gov/grants/.../guide-for-the-care-and-use-of-laboratory-animals.pdf) must be met for all rodents until time of euthanasia.

- Euthanasia of more than one animal at a time should always be performed in cohorts of live animals (i.e., live animals must not be placed in the chamber with dead animals).

To reduce stress, rodents should be euthanized in their home cage whenever possible. This can be accomplished by delivering CO₂:

a. Via a Euthanex® lid over the home cage after removing the filter and wire tops.
b. Via the air supply port of an IVC cage.

Alternatively, animal(s) are placed in a clean micro-isolator chamber with a fitted lid.

CO₂ delivery to the cage/micro-isolator chamber is accomplished by turning the CO₂ cylinder valve and flow meter on so that animal(s) are slowly exposed to increasing levels of CO₂. An optimal flow rate for CO₂ euthanasia systems should displace 10% to 30% of the chamber or cage volume/min. Prefilled chambers are unacceptable.
Flowmeter Calculation Instructions
(For Tank Chambers of varying size)

The flow meter allows accurate filling of the chamber volume at any given flow rate of CO₂ per minute. However, percent flow rate must first be converted to liters/minute (L/min) and will depend on the size of the chamber. To make the conversion from 20% flow rate per minute to L/Min, the following calculation is used:

1. Determine the volume of the euthanasia chamber in liters by measuring its length, width, and height in centimeters (cm).
2. Multiply these dimensions, (length x width x height) to obtain the volume in cubic centimeters (cm³).
3. Convert volume in cm³ to volume in liters (1 cm³ = 0.001 liters so multiply volume in cm³ by 0.001 to get volume in liters).
4. Multiply volume of cage in liters by 20% flow per minute.
5. If the flow meter is calibrated for air, divide by 0.812, the conversion factor for CO₂.

Flow Rates for Standard Cages

<table>
<thead>
<tr>
<th>Species</th>
<th>Cage Type</th>
<th>Flow Rate (L/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
<td>Static (Small Shoebox)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Static (Large Shoebox)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Disposable</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>IVC (M.I.C.E.)</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>IVC (Allentown)</td>
<td>1.6</td>
</tr>
<tr>
<td>Rats</td>
<td>Static</td>
<td>8</td>
</tr>
</tbody>
</table>

Stress and Pain Assessment

There are 4 stages of anesthesia prior to death. Stage I is the period from induction to loss of consciousness. Stage II extends to loss of response to stimuli (excitatory phase). Stage III shows muscle relaxation, amnesia, and analgesia to stimuli (surgical phase). Stage IV extends to cessation of life functions including respiratory and finally cardiovascular systems.

Progressing from stage I through IV as quickly and smoothly as possible is the goal for humane euthanasia.
Additional Considerations

- Gas flow may be increased as loss of consciousness is observed and flow should be maintained for at least one minute after apparent clinical death (e.g., cessation of cardiovascular and respiratory movements).
- Turn off CO₂ cylinder valve and flow meter when the euthanasia process is complete.
- Chambers/lids must be cleaned/dried between animals or groups of animals to minimize odors that might distress animals prior to euthanasia.
- Animals must not be euthanized in animal housing rooms except during special circumstances such as during quarantine and/or exposure to infectious agents on a case by case basis.
- Death must be verified after euthanasia and prior to disposal. Unintended recovery must be prevented by the use of appropriate CO₂ concentrations and exposure times, and by use of a secondary physical method. The recommended secondary physical methods are:
  1. Decapitation
  2. Perfusion of a histological fixative via the major blood vessels
  3. Pneumothorax by opening the thorax
  4. Complete severing of the spine just below the base of the skull using a dorsal approach
  5. Cervical dislocation for animals under 200g.

It is important that you understand that unintended recovery of animals after apparent death from CO₂ (e.g., in a necropsy cooler, morgue cooler) is a serious noncompliance issue. It will be reported to OLAW and could result in loss of privileges or funding.

If you are uncertain of any aspect of the euthanasia protocol or have questions please confer with the Animal Care Staff before proceeding. They are there to assist and ensure animals are properly and humanely euthanized.

REFERENCES


Guide for the Care and Use of Laboratory Animals, 8th Edition (2011)

Public Health Service Policy on Humane Care and Use of Laboratory Animals (2002)