CARBON DIOXIDE EUTHANASIA

PURPOSE:

Describes the procedure for using carbon dioxide as a means of euthanasia for adult small animals (for e.g., mice, rats, other small rodents). This protocol is not to be used for rabbits.

POLICY:

This method is recommended for small animals only. Filling the euthanasia chamber at a rate of 20-30% CO₂ chamber volume per minute has been shown to cause the least amount of distress to rodents. Euthanizing rodents using a much higher flow rate or pre-filling the chamber is known to cause both pain and distress to the animals. Therefore, pre-filling the chamber is not acceptable. A flowmeter should be used to accurately fill the chamber at the appropriate flow rate.

RESPONSIBILITY:

Investigator, technicians, veterinarian

MATERIALS:

- Euthanasia chamber (preferably “home cage”)
- CO₂ tank (100% CO₂) fitted with appropriate flow gauge, pressure regulator and flowmeter

PROCEDURES:

1. Use the animal’s home cage or if that is not possible, use a clear empty polycarbonate box appropriately sized for the species.
2. Do not mix animals from different cages as this may cause unnecessary stress. Do not overfill cages with animals; i.e., use the appropriate number of animals for the particular cage size.
3. Remove home cage lid and cover with a clear plastic top with a hole made for the CO₂ tubing or place animals gently into new cage with clear plastic top.
4. Turn on the CO₂ at a low flow rate (20-30% of the volume of the cage per minute; see below for calculations) and leave CO₂ running until animals stop breathing.
5. Turn off the CO₂. Leave the lid on to expose the animals to CO₂ gas for another 5 minutes. Animals may be left in chamber for approximately 30 minutes to assure death.
6. At this point, heartbeat and respiration should be checked to verify death.
7. CO₂ euthanasia should be followed by one of the following forms of physical euthanasia before animals are placed in bags for disposal:
   - Bi-lateral pneumothorax
   - Removal of the heart
   - Cervical dislocation
   - Decapitation

   (Under no circumstances, except those approved by the Animal Care Committee, should these procedures be performed on animals not already euthanized with CO₂.)
8. Dead animals should then be properly disposed of.

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USE OF A FLOWMETER:

The flowmeter allows you to accurately fill the chamber volume at any given flow rate of CO\textsubscript{2} per minute; however % 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, for e.g., you can make the following calculation:

1. First, you must know the volume of the euthanasia chamber in liters (L). To do this, measure the length, width and height of your chamber in centimeters (cm) and multiply all together to get the volume in cm\textsuperscript{3}. 1 cm\textsuperscript{3} is equal to 1 millimeter (ml); then convert to liters (L) by dividing by 1000.

   Example: Cage dimensions are L 28 cm x W 17 cm x H 21 cm ≈ 10000 cm\textsuperscript{3} = 10000 ml = 10 L

2. Multiply volume of cage (L) by 20% flow per minute

   Example: 10 L cage x 0.20 = 2 L/min

3. If your flowmeter is calibrated for air, you must divide flow rate by a conversion factor for CO\textsubscript{2} which is 0.812

   Example: 2 L/min ÷ 0.812 = 2.5 L/min

Therefore, for 20% CO\textsubscript{2} flow rate, set your flowmeter to 2.5 L/min when using a 10 L cage.

REFERENCES:

Canadian Council on Animal Care: www.ccac.ca

AVMA: www.avma.org/resources/euthanasia.pdf