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% CO_2 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")
- CO_2 tank (100% CO_2) 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 CO_2 tubing or place animals gently into new cage with clear plastic top.
4. Turn on the CO_2 at a low flow rate (20-30% of the volume of the cage per minute; **see below for calculations**) and leave CO_2 running until animals stop breathing.
5. Turn off the CO_2. Leave the lid on to expose the animals to CO_2 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. CO_2 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 CO_2.)

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_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^3. 1 cm^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^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_2 which is 0.812

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

Therefore, for 20% CO_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

American Veterinary Medical Association (2001) 2000 Report of the AVMA Panel on Euthanasia. Journal of the Veterinary Medical Association, 218(5): 669-696.