UBC Animal Care Guidelines.
SOP: ACC-2011- 01
Submitted by: UBC Animal Care Committee
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SOP: Carbon Dioxide Euthanasia of Neonatal Rodents

PURPOSE: Describes the procedure for using carbon dioxide as a means of euthanasia for neonatal rodents less than 10 days of age (e.g., mice, rats, other small rodents). This protocol is not to be used for rabbits.

POLICY: This method is recommended for rodents under 10 days of age only, but **based on CCAC's conditional approval of C02 euthanasia of conscious animals, another acceptable form of euthanasia should always be considered first**. 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 adult 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 flow meter should be used to accurately fill the chamber at the appropriate flow rate.

RESPONSIBILITY: Investigators, technicians, facility staff

REFERENCES: CCAC Guidelines on: Euthanasia of Animals Used in Science www.ccac.ca

MATERIALS:

- Euthanasia chamber (solid clear cage or container appropriate to the size of the animals)
- Clear plastic bags (5 to 10L volume, new with no holes)
- CO2 tank (100% CO2) fitted with appropriate flow gauge, pressure regulator and flow meter
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PROCEDURES:

1. Use a clear empty polycarbonate box (mouse cage or other) without a lid, appropriately sized for the species. Open a clear plastic bag and set the polycarbonate box inside the bag.

2. Place the litter or litters gently into the box with their nesting material. Animals should not be piled and should be able to perform normal postural movements.
3. Gather the plastic bag excess around the CO2 tank hose, on top of the container. Avoid placing the hose near the pups as pressurized C02 can be very cold.

4. Turn on the CO2 at a low flow rate (30% of the volume of the bag per minute; **see below for calculations**) and leave CO2 running until the bag is visibly full.
5. Turn off the CO2. Quickly secure the bag by tying the excess or using a twist tie. Note that the tie must prevent the leakage of CO2 so it must be tied very tightly.

6. The cage should be kept warm as this will hasten the euthanasia of pups. A heating pad on low may be placed under the cage but animals being provided heat should never be left unattended. As neonates are very resistant to carbon dioxide, death may take 20 minutes to 1 hour depending on the age of the pups.
7. Pups should be checked regularly (i.e. every 10 minutes) during exposure to C02 to ensure that no complications occur, such as loss of CO2 from the bag.
8. Once pups have stopped moving including respiration, **all pups must be decapitated using sharp scissors** to ensure death prior to disposal.

USE OF A FLOWMETER:

1. The flowmeter allows you to accurately fill the chamber or bag volume at any given flow rate of CO2 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 30% 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 bag in **liters (L)**. To do this, measure the length, width and height of your bag in centimeters (cm) and multiply all together to get the volume in cm3. 1 cm3 is equal to 1 millimeter (ml); then convert to liters (L) by dividing by 1000.
   Example: Bag dimensions are L 28 cm x W 17 cm x H 21 cm ≈
   - 10000 cm3 = 10000 ml = 10 L
2. Multiply volume of bag (L) by 30% flow per minute **Example**: 10 L cage x 0.30 = 3 L/min
3. If your flowmeter is calibrated for air, you must divide flow rate by a conversion factor for CO2 which is 0.812 **Example**: 3 L/min ÷ 0.812 = 3.69 L/min

2. Therefore, for 30% CO2 flow rate, set your flowmeter to 3.69 L/min when using a 10 L bag.