

UBC Animal Care Guidelines
SOP: ACC CCM 2012-03 Euthanasia of Adult Rodents
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EUTHANASIA OF ADULT RODENTS USING AN INHALANT ANESTHETIC, FOLLOWED BY CARBON DIOXIDE

PURPOSE:

To describe the procedure for using an inhalant anesthetic, followed by carbon dioxide as a means of euthanasia for adult rodents

POLICY:

This method is recommended for adult rodents only. Using only CO₂ to euthanize rodents is a conditionally acceptable method of euthanasia (CCAC guidelines) since CO₂ euthanasia of conscious animals is known to cause distress +/- pain. Therefore, to render an animal unconscious using inhalant anesthetics first is preferred to using CO₂ solely.

RESPONSIBILITY:

Those trained persons listed on an approved Animal Care Committee protocol performing the procedure

MATERIALS:

- Euthanasia chamber (preferably "home cage") (can be appropriately sized anesthetic induction chamber)
- Calibrated inhalant anesthetic vaporizer and inhalant anesthetic (for e.g., isoflurane)
- Oxygen tank
- Scavenging system for waste gases (fume hood, fully ducted BSC, down draft table, etc.)
- CO₂ tank (100% CO₂) fitted with appropriate flow gauge, pressure regulator and flow meter

PROCEDURES:

1. Use the animal's home cage or if that is not possible, use a clear empty polycarbonate box (or anesthetic induction chamber) 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/chamber size.
3. Attach the anesthetic delivery circuit to the euthanasia chamber.
4. Turn on the oxygen flow rate. For small cages or chambers around 2 Liters (L) in volume, use 1-2 L oxygen/minute. For larger cages or chambers, use 3-4 L oxygen/minute.
5. Turn on the vaporizer to the highest setting (5%) until the animals are recumbent and immobile, and their breathing is deep and slow (approx. 60-90 seconds).
6. If using a dual inhalant/CO₂ anesthetic machine, turn off the anesthetic and turn the CO₂ on. A very high flow rate should be used (i.e., 3-4 times of 20-30% gradual flow rate for conscious rodents, so for example, if euthanizing a conscious rodent without anesthesia using a 10 L volume cage, one would normally use a flow rate of 20% or 2 L/min---if the animal is anesthetized first, use a flow rate 3-4 times higher or 6-8 L/min).**
7. If a dual inhalant/CO₂ machine is not available, you can still use the same euthanasia chamber for both inhalant anesthetic and CO₂---so for example, if the animal is in an induction chamber or its home cage and being anesthetized with isoflurane, once it is unconscious, turn off the anesthetic vaporizer and oxygen flow. Then insert the tube delivering CO₂ into the same euthanasia chamber without transferring the animal from a chamber containing isoflurane to one containing CO₂. This should facilitate the transition of gases and minimize exposure to waste gases, and prevent the animal from recovering from the anesthetic.
8. Observe animal until it stops breathing. Turn off the CO₂. Leave the lid on the chamber or cage to continue exposure of the animals to CO₂ gas for at least another 5 minutes. Animals may be left in the chamber for approximately 30 minutes to assure death.
9. Appropriately vent and scavenge waste gases prior to opening the chamber.
10. At this point, heartbeat and respiration should be checked to verify death.
11. Inhalant/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 physical procedures be performed on animals not already euthanized.)

8. Dead animals should then be properly disposed of.

**The reason a lower flow rate (for e.g., 20% flow rate---about 2 L/minute in a shoebox sized mouse cage) is used if the animal is conscious, is because when it is awake, it will feel distress (but not pain) since it will become unconscious before the CO₂ concentration reaches levels that cause pain, but if a high flow rate is used, the CO₂ levels rise quickly and the animal will experience both pain and distress. If an animal is anesthetized first, once it is switched over to CO₂, the CO₂ flow rate can and should be turned up very high. The animal is already anesthetized, so is unconscious (therefore, cannot feel the distress/pain associated with high CO₂ flow rates)---since you do not want it to regain consciousness, and you want it to die quickly, a very high flow rate is used.

IMPORTANT NOTE: Inhalant anesthetics should only be used when appropriate scavenging is available.

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.