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| University Animal Care Committee Standard Operating Procedure | | |
| Document No: 7.5.2 | Subject: CO ₂ Euthanasia for Mice | |
| Date Issued: August 21, 2012 | Revision: 3 | Page No: 1 |

Location: Queen's University

Responsibility: Principal Investigators (PI), Research Staff, Veterinary Staff

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe the procedure for the euthanasia of adult rodents using CO₂ asphyxiation.

1. Introduction and Definitions: As per the UACC policy on euthanasia of animals used in science, CO₂ euthanasia is a *conditionally acceptable* method (current Canadian Council on Animal Care guidelines). Principal Investigators will be required to justify its use (within their animal use protocol). Following euthanasia with CO₂ asphyxiation, a secondary method must be used to confirm death.

2. Materials:

- CO₂ source (facility outlet, or tank and regulator)
- Flow meter
- Appropriately sized lid for the rodent cage (custom lids for CO₂ euthanasia)
- Instruments (for secondary method of euthanasia)
- Body bags

3. Procedures:

- To minimize stress, animals should be euthanized in their home cage with a maximum of:
 - Five adult mice or one litter per cage (do not pool mice from different cages).
 - Two adult rats or one litter per cage (do not pool rats from different cages).
 - Neonatal mice (up to 10 days of age) are resistant to the hypoxia induced by CO₂; therefore, alternative methods are recommended (e.g., decapitation using sharp blades).
- Place the appropriately sized CO₂ lid on the animal cage (remove cage lid first). Lid holes must be unobstructed and over the body of the cage.
- Ensure the regulator hose is connected to the lid fitting.
- Do not pre-charge the chamber.
- Open the CO₂ tank valve (if using).
- Set the regulator to the appropriate setting (35% Displacement):
 - Standard mouse cage (5" x 8" x 15"): 3.44 LPM (liters per minute)
 - Standard rat cage (7.5" x 13.5" x 14.75"): 8.6 LPM (liters per minute)
 - Double decker rat cage (10" x 18" x 14.5"): 15.0 LPM (liters per minute)
 - Cages of different dimensions:
 - Measure the cages width, length and height and multiply them to determine the volume in cubic inches.
 - Then divide this by 61 to convert into liters and multiply by 35% to determine flow rate:
 - height x width x length = liters X 35% = flow rate



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- After the animals have become unconscious, the flow rate can be increased to minimize the time of death. The time required for euthanasia can be several minutes.
- Maintain the CO₂ flow until the animal has stopped breathing.
- Close the flow meter, or the valve on the tank (if using tank system).
- Leave the animals in contact with loaded CO₂ cage for a minimum of 2 additional minutes.
- To confirm death, monitor animals for the following signs: no chest movement, no palpable heartbeat, poor mucous membrane color, no response to toe pinch, color change or opacity of the eyes.
- Once the cage is empty and the session is complete, ensure the flow is off, and flush the line (if using this system).

Note: A secondary method of euthanasia such as cervical dislocation or bilateral pneumothorax is required for your animals (unless justified in the animal use protocol) before disposal to ensure death.

Euthanasia of Fetal and Neonatal Rodents:

When ovarian hysterectomies are performed, euthanasia of fetuses should be accomplished as soon as possible after removal from the dam. Neonatal animals are resistant to hypoxia, and as all inhalant agents lead to hypoxia, neonatal animals take longer to die than adults. It is recommended that inhalant agents not be used alone in animals less than 16 weeks old, except to induce loss of consciousness, followed by the use of some other method to kill the animal.

Euthanasia of Fetuses up to 15 Days Gestation:

- Neural development during this developmental stage is minimal and pain perception is considered unlikely. Euthanasia of the mother for removal of the fetus should ensure rapid death of the fetus due to loss of blood supply and non-viability of fetuses at this stage of development.

Over 15 Days Gestation:

- Approved methods of euthanasia of fetuses includes skillful injection of chemical anesthetics in sufficient quantities to ensure death, or decapitation with sharp surgical/decapitation scissors, or cervical dislocation.
- When chemical fixation of the whole fetus is required, fetuses should be anesthetized prior to immersion in, or perfusion with, fixative solutions. Anesthesia may be induced by hypothermia, or by injection with a chemical anesthetic.
- Rodent fetuses are resistant to hypoxia. Near-term rodent fetuses experiencing umbilical cord occlusion exhibited respiratory movements for up to 40 min after occlusion (as per the ACLAM Task Force on Rodent Euthanasia). Fetuses require extended exposure to inhalant anesthetics, including CO₂.



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- When fetuses are not required for study, the method chosen for euthanasia of a pregnant mother should ensure cerebral anoxia to the fetus and minimally disturb the uterine milieu to minimize fetal arousal. A recommended method for euthanasia of the mother in this circumstance is CO₂ exposure followed by cervical dislocation.

Euthanasia of Neonates:

Neonates up to 10 Days of Age

- Maturation of nociceptors and the development of excitatory and inhibitory receptor systems occur during the period just prior to birth and extend into the 2 week of postnatal life.
- Resistance to hypoxia results in a prolonged time to unconsciousness when CO₂ inhalation is used as a euthanasia agent. The duration of exposure to carbon dioxide varies with the age of the neonate. Inbred and outbred neonatal mice less than 7d of age may differ in susceptibility to CO₂, requiring exposures as long as 50 min to ensure euthanasia. When using CO₂ for euthanasia, death must be verified prior to disposal of the carcass and a physical secondary method is required unless justified within the protocol.
- Methods for the euthanasia of neonatal mice includes injection of chemical anesthetics in sufficient quantities to ensure death, (scissor) decapitation or cervical dislocation.

Neonates over 10 Days of Age

- Follow guidelines for adults.

References:

[Journal of the American Association for Laboratory Animal Science](#), Volume 45, Number 1, January 2006, pp. 98-105(8) James Artwohl, Patricia Brown, Brian Corning and Susan Stein Report of the ACLAM Task Force on Rodent Euthanasia

<https://www.ccac.ca/Documents/Standards/Guidelines/Euthanasia.pdf>

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