University Animal Care Committee Standard Operating Procedure		
Document No: 7.5.1	**Subject:** Euthanasia Method for Rodents via Barbiturate or Injectable Anaesthetic	
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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 rodents using an overdose of barbiturates or injectable anaesthesia.

1. **Introduction and Definitions:** As per the UACC policy on euthanasia of animals used in science, overdose of barbiturates or injectable anaesthetic is an acceptable method of euthanasia for rodents when conducted properly. Unless justified and approved in the Animal Use Protocol, a Primary method (rendering death) of euthanasia must be followed by a Secondary (confirmation of death) method.

2. **Materials:**
   - Sterile needles (assorted sizes – 23-27g)
   - Sterile syringes (assorted sizes 1-10ml)
   - Barbiturate or injectable anaesthesia

3. **Procedures:**
   - Using an appropriate gauge needle, restrain the animal gently and inject three times the calculated anesthetic dose or 100mg/kg sodium pentobarbital intraperitoneally.
   - Following the injection, the animal should be placed back in its cage (lined with paper to avoid inhalation of bedding material), and placed in a quiet area to minimize excitement and trauma until euthanasia is complete. Multiple animals should not be placed within the same cage during anesthesia.
   - To confirm death, monitor the animal for the following signs: cessation of chest movement, no palpable heartbeat, poor mucous membrane color, no response to toe pinch, and color change/opacity of the eyes.
   - Carcasses should be disposed of promptly.

****A physical method of euthanasia such as a bilateral pneumothorax, cervical dislocation, or exsanguination is required before disposal of the animal to confirm euthanasia. The handler must ensure complete separation between the cervical vertebrae and skull (severance of the spinal cord) if cervical dislocation is the secondary method.****

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 CO_2.

- 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 CO_2 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

CO_2, requiring exposures as long as 50 min to ensure euthanasia. When using CO_2 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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