

University Animal Care Committee Standard Operating Procedure		
Document No: 7.5.5	Subject: Euthanasia Method for Rodent Neonates and Fetuses	
Date Issued: March 7 th , 2023	Revision: 2	Page No: 1

Location: Queen's University

Responsibility: Principal Investigators, Research Staff, Veterinary Staff

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe the procedure for the euthanasia of rodent neonates and fetuses.

1. Introduction and Definitions: As per the UACC policy on euthanasia of animals used in science, there are several acceptable methods of euthanasia for neonate and fetal rodents.

Abbreviations: Animal Care Services **ACS**, Principal Investigator **PI**, subcutaneous **SC**, intravenous **IV**, intraperitoneal **IP**, intramuscular **IM**, per os **PO**, per rectum **PR**

2. Materials:

- Calibrated inhalant isoflurane anaesthetic vaporizer with anaesthetic induction chamber
- Oxygen
- Instruments for secondary method (e.g. scissors, cage card holder)
- Injectable Anesthetics

3. Procedures:

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 ensures rapid death of the fetus due to loss of blood supply.¹

Over 15 Days Gestation:

- 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

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extended exposure to inhalant anesthetics, including CO₂. 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.

- When fetal tissue is required, euthanasia includes skillful injection of chemical anesthetics in sufficient quantities to ensure death, or decapitation with sharp surgical/decapitation scissors.
- 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.

Euthanasia of Neonates:

Neonates up to 14 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
- Methods for the euthanasia of neonatal mice includes scissor decapitation or intraperitoneal injection of an anesthetic followed by a physical means of euthanasia.
- 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 7 d 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.

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Neonates over 14 Days of Age

- Between the age of 14 days and weaning, mice can be euthanized with isoflurane, followed by a secondary method.
- Isoflurane anesthetic chambers should not be overloaded and need to be kept clean to minimize odors that might distress the next animal euthanized.
- The anesthetic can be introduced at a high concentration from a vaporizer of an anesthetic machine connected to an adequate scavenging system or air filter.

Mouse Age	Method of Euthanasia
Embryo – E15	<ul style="list-style-type: none"> • Euthanasia of the Dam
E16 – Birth (No Fetal Tissue required)	<ul style="list-style-type: none"> • Euthanasia of the Dam
E16 – Birth (Fetal Tissue or Whole Fetus required)	<ul style="list-style-type: none"> • Dam is euthanized, fetus will be euthanized by scissor decapitation or injectable anesthetic ² • If whole Fetus is required, injectable anesthetic or hypothermia required before fixative.
Birth – 14 days (P14)	<ul style="list-style-type: none"> • Scissor decapitation OR • Injectable anesthetic (IP)²
Neonates 15 days (P15) – 21 days (P21)	<ul style="list-style-type: none"> • Inhalant overdose ² OR • Injectable anesthetic (IP) ²

¹ Note: For dam euthanasia methods, please refer to:

- SOP 7.5.1 – Euthanasia of Adult Rodents via Barbiturate or Injectable Anesthetic Overdose
- SOP 7.5.2 – Euthanasia of Adult Rodents via CO₂ Asphyxiation
- SOP 7.5.3 - Euthanasia of Adult Rodents via Overdose of Inhalant Anesthesia
- SOP 7.5.4 – Euthanasia of Rodents via Physical Methods

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²Note: A physical method of euthanasia such as cervical dislocation, bilateral pneumothorax, heart snip or exsanguination is required (unless otherwise justified in the animal use protocol) before disposal to ensure death.

References:

[Journal of the American Association for Laboratory Animal Science](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1360333/), 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>

SOP Revision History:

Date	New Version
1/22/2024	Added a chart with acceptable euthanasia methods
2/21/2024	Updated chart and wording
3/26/2025	Updated injection method of euthanasia