



University Animal Care Committee Standard Operating Procedure		
Document No: 7.5.3	Subject: Euthanasia Method for Adult Rodents via Overdose of Inhalant Anaesthesia	
Date Issued: August 21, 2012	Revision: 1	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 overdose of inhalant anaesthesia.

1. **Introduction and Definitions:** As per the UACC policy on euthanasia of animals used in science, overdose of inhalant anesthesia is an acceptable method of euthanasia for adult rodents when conducted properly.

2. Materials:

- Bell jar containing gauze/cotton soaked with an appropriate inhalant anaesthetic (isoflurane)
- Calibrated inhalant isoflurane anaesthetic vaporizer with anaesthetic induction chamber
- Oxygen
- Instruments for secondary method (e.g. scissors, cage card holder)

3. Procedures:

- Anesthetic chambers should not be overloaded and need to be kept clean to minimize odors that might distress the next animal euthanized.
 - The animal can be placed in a closed receptacle (bell jar) containing cotton or gauze soaked with an appropriate amount of the anesthetic, provided it is not in direct contact with the animal (grid or mesh should be used to separate gauze from rodent). The liquid state of most inhalant anesthetics is irritating; animals should be exposed only to vapors. Procedures should be conducted in a chemical fume hood to prevent inhalation of the anesthetic by personnel.
 - The anesthetic can also be introduced at a high concentration from a vaporizer of an anesthetic machine connected to an adequate scavenging system or air filter.
 - Sufficient room air or O₂ must be provided during the induction period to prevent hypoxemia. In the case of small rodents placed in a closed receptacle (bell jar), there is sufficient O₂ in the chamber to prevent hypoxemia short-term.
 - Neonatal animals (up to 10 days of age) are resistant to the hypoxia induced by high anesthetic gas concentrations, therefore, alternative methods are recommended. Inhalant anesthetics may be used for narcosis of neonatal animals provided it is followed by a secondary method of euthanasia (e.g. decapitation using sharp blades).
 - To confirm death, monitor animal for the following signs: no chest movement, no palpable heartbeat, poor mucous membrane color, no response to toe pinch, color change or opacity in eyes.
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University Animal Care Committee Standard Operating Procedure		
Document No: 7.5.3	Subject: Euthanasia Method for Adult Rodents via Overdose of Inhalant Anaesthesia	
Date Issued: August 21, 2012	Revision: 1	Page No: 2

Note: 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.

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 appear to be resistant to hypoxia, and because all inhalant agents ultimately cause 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. 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
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University Animal Care Committee Standard Operating Procedure		
Document No: 7.5.3	Subject: Euthanasia Method for Adult Rodents via Overdose of Inhalant Anaesthesia	
Date Issued: August 21, 2012	Revision: 1	Page No: 3

- 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.
- 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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