



University Animal Care Committee Standard Operating Procedure		
Document No: 10.5.4	Subject: Euthanasia of Rodents via Physical Methods (Rat)	
Date Issued: August 21, 2012	Revision: 2	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 approved techniques for the euthanasia of rodents using physical methods.

- 1. Introduction and Definitions:** As per the UACC policy on euthanasia of animals used in science, physical methods of euthanasia are considered conditionally acceptable. As a result, the method of euthanasia must be scientifically justified by the Principal Investigator and approved by the University Animal Care Committee.

Physical methods must only be used by highly competent individuals. As per the CCAC, some of these methods for killing experimental animals may be acceptable for use in certain circumstances where there is scientific justification, following review and approval by the UACC and assurance that trained personnel are available. These are not considered 'acceptable methods', but rather "conditionally acceptable" because there is greater potential for operator error or safety hazards; they might not consistently produce humane death, or they are not well documented in the scientific literature. When conditionally acceptable methods (physical methods) that have been approved by the UACC are used, the conditions of use and training of the personnel involved should be clearly stated in the protocol.

2. Materials:

- A clean blunt object such as a metal cage card holder, scalpel handle, scissors, etc.
- Commercial cervical dislocator/luxator
- Guillotine
- Plastic cones (decapicones)
- Decapitation scissors
- Various sizes of sterile needles and syringes
- Body bags

3. Procedures:

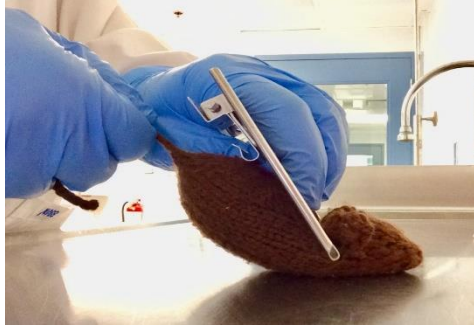
***Unless described in the animal use protocol and approved by the UACC, all physical methods of euthanasia must be performed under anaesthesia following the appropriate anaesthesia SOP (10.6 Anesthesia in Rats). The UACC requires that a Primary (rendering death) and Secondary (confirmation of death) method of euthanasia be described on all animal use protocols (unless justified and approved). ***

- All personnel involved in the euthanasia of laboratory animals must be trained in the procedures listed on the protocol, and in the proper and safe use of the equipment.
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Cervical dislocation:

- For rats under 200g, the thumb and index finger are placed on either side of the neck at the base of the skull or, alternatively, a rod/cage card holder is pressed at the base of the skull. With the other hand, the base of the tail or the hind limbs are quickly pulled, causing separation of the cervical vertebrae from the skull. **The handler must ensure that they feel for complete separation between the cervical vertebrae and skull prior to disposal.**
- For rats under 200g, position a cage card holder (or rod, scalpel handle, scissor blade, etc.) at the base of the occipital bone, angled cranially ~45°. With the other hand, grasp the base of the tail and elevate the animal's body at a similar angle to the device. The user must then push forward and down with the cage card holder, while pulling back on the base of the tail. A commercial device may also be used.
- Alternatively, position the thumb and index finger on either side of the neck at the base of the skull (behind the occipital bone). With the other hand, holding the base of the tail, quickly and solidly pull causing separation of the cervical vertebrae from the skull.



<http://oslovet.norecopa.no/teaching/mouse/techniques/rederive/default.html>

Training for this technique is required to ensure that dislocation is cervical and not lower in the vertebral column. The effectiveness of dislocation must be verified by feeling for a separation of cervical tissues. When the spinal cord is severed, an approximate 5mm space will be palpable between the occipital condyles and the first cervical vertebra. If adequate separation is not observed, a backup method, such as decapitation, bilateral pneumothorax, or a heart snip/removal should be used immediately.

Decapitation:

- Decapitation is often used when chemical methods may interfere with research results. When decapitation is used, the blades should be kept very sharp and guillotines should be well maintained and cleaned between uses to prevent transmission of olfactory clues.



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- Guillotines that are designed to accomplish decapitation in adult rodents in a uniformly instantaneous manner are commercially available.
- The use of plastic decapicones to restrain animals is strongly recommended as it reduces distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal in the guillotine.
- Guillotines are not commercially available for neonatal rodents, but sharp blades (e.g. scissors) can be used for this purpose.
- The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades.
- Decapitation of young rats by scissors may be approved in an Animal Use Protocol, however sharp surgical scissors or decapitation scissors are mandatory. Anesthesia is also a requirement and must be described in the AUP.

Exsanguination:

- Animals may be exsanguinated to harvest large volumes of blood, but only when deeply anesthetized (surgical plane).
- Deeply anesthetize animal according to SOP 10.6 “Anesthesia in Rats”.
- Collect blood from the heart via cardiac puncture technique.
- To confirm death, monitor animal for the following signs: no rising and falling of chest, no palpable heartbeat, poor mucous membrane color, no response to toe pinch, color change in eyes.
- A physical method of euthanasia, such as cervical dislocation or bilateral pneumothorax, is required immediately afterwards to ensure death.

Cardiac Perfusion:

- *Described in a stand-alone SOP (10.15).*

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.
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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.
- 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 rats 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 rats 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.ccaac.ca/Documents/Standards/Guidelines/Euthanasia.pdf>

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