

SOP #: 301.01

Page: 1 of 5

Rodent Euthanasia

The intent of this Standard Operating Procedure (SOP) is to describe the different recognized methods for euthanasia of rodents at NUS Comparative Medicine (CM). This SOP is intended for all staff and personnel carrying out euthanasia of rodents. This procedure is approved by the Institutional Animal Care and Use Committee (IACUC). Any deviation must be approved by the IACUC prior to its implementation

TABLE OF CONTENTS

1. Introduction
2. Materials
3. Procedures
4. Personnel Safety
5. Animal Related Contingencies
6. References

1. INTRODUCTION

Euthanasia is the act of humanely sacrificing animals by methods that induce rapid consciousness and death without pain or distress. It may be planned or necessary at the end of a protocol, or as a means to relieve pain or distress that cannot be alleviated by analgesics, sedatives, or other treatments.

This SOP provides guidelines for the humane euthanasia of rodents using several techniques.

2. MATERIALS

- a. Euthanasia equipment
 - i. Euthanasia chamber with CO₂ gas supply
 - ii. Barbiturates/Euthanasia solution
 - iii. Decapitation device (i.e. guillotine) or dedicated scissors/blades
- b. Support supplies
 - i. Needles and syringes
 - ii. Sharp container
 - iii. Bag or container for animal carcass disposal

3. PROCEDURES

- a. General requirements

The procedures should be performed only by personnel validated by the CM Vet staff of their competency in performing the procedures.

- ii. Animals should be euthanized in a quiet, clean environment, and preferably away from other animals.
- iii. Carcass must not be disposed until death is established.
- iv. Adhere to IACUC-approved protocols.
- v. Maintain equipment to ensure optimal performance.

b. Non-physical Method

i. Carbon Dioxide Inhalation:

1. Compressed CO₂ gas in cylinders is the only acceptable source of CO₂.
2. Place the animal(s) in a dedicated euthanasia chamber or rodent cage. Do not overcrowd the chamber. Use a chamber large enough to permit each animal to stand on the floor of the chamber with all four feet and have sufficient space to turn around and perform normal postural adjustments.
3. Connect the compressed CO₂ gas cylinder to the euthanasia chamber via a hose and dedicated chamber lid, or by passing a hose through a lixit hole or similar opening.
4. Open the tank valve and set the flow so to displace at least 20% of the chamber volume per minute (usually ~5 L/min) to induce rapid unconsciousness with minimal distress to the animals.
5. Maintain gas flow for at least 1 minute after respiration has ceased.

Important: Verify that an animal is dead before removing it from the chamber by making sure there is no respiratory movement for at least 3 minutes. If the animal is not dead, follow the CO₂ administration by another method of euthanasia (e.g. cervical dislocation, bilateral thoracotomy).

ii. Overdose of inhalant anesthetic (isoflurane)

Note: This method is not approved as a sole mean of euthanasia for neonates up to 10 days of age; see section on Euthanasia of neonates.

1. Expose the animal to a high gas concentration using an anesthetic vaporizer.
2. Vapors are inhaled until respiration ceases and death ensues.

Important: Verify that an animal is dead before disposing of the carcass, by making sure there is no respiratory movement for at least 3 minutes. If the animal is not dead, follow the anesthesia by another method of euthanasia (e.g. cervical dislocation).

iii. Overdose of injectable barbiturate (pentobarbital)

1. Inject >150 mg/kg of pentobarbital euthanasia solution at recommended dosage intraperitoneal (IP).

Important: Verify that an animal is dead before disposing of the carcass, by making sure there is no respiratory movement for at least 3 minutes. If the animal is not dead, follow the injection by another method of euthanasia (e.g. cervical dislocation, bilateral thoracotomy).

c. Physical methods

i. Considerations

1. Use these techniques only when scientifically justified and approved by the IACUC.
2. Apply prior anesthesia or sedation whenever possible.
3. If anesthesia is contraindicated, these methods can be applied only by an individual whose proficiency is validated by CM veterinary staff; documentation of proficiency is maintained in the CM training log database.

ii. Cervical dislocation

Note: This method can be applied to rodents weighing less than 200 g (if not under deep anesthesia).

1. Place the thumb and index finger on either side of the neck or at the base of the skull, or alternatively, press a rod at the base of the skull
2. With the other hand, quickly pull the base of the tail or the hind limbs, causing separation of the cervical vertebra from the skull.
3. Confirm separation by palpation of the cervical region.

iii. Decapitation

Guillotines that are designed to accomplish decapitation in adult rodents in a uniformly instantaneous manner are commercially available. For neonatal rodents, guillotines are not commercially available but sharp blades or 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. A log must be kept that indicates when the equipment was checked and by whom.

1. Use an appropriately sized guillotine. Decapitate neonates using scissors or scalpel.
2. Quickly separate the head from the body at the cervical level.

NOTE: This technique is conditionally acceptable if performed correctly, and it should be used in research settings when its use is required by the experimental design and approved by the IACUC.

iv. Exsanguination

1. Deeply anesthetize the animal, as per SOP# 101 “Rodent Anesthesia”.
2. Verify that withdrawal reflex is absent by pinching the toes.
3. For cardiac puncture, insert a needle (~23 G) at a 30° angle to the left junction formed by the sternal appendix and the last rib.
4. For abdominal aorta puncture, incise the abdomen and retract viscera to expose the aorta. Insert a needle (~23 G for the rat, 25 G for the mouse) into the vessel.
5. Withdraw the maximal volume of blood (~1 mL for the mouse, and ~10 mL for the rat).

Important: Verify that an animal is dead before disposing of the carcass, by making sure there is no respiratory movement for at least 3 minutes. If the animal is not dead, follow the exsanguination by another method of euthanasia (e.g. cervical dislocation, bilateral thoracotomy).

d. Euthanasia of neonates

Generally, neonatal rodents are more resistant to hypoxia than adults of the same species. CO₂ delivery needs to be prolonged for up to 10 minutes or longer to assure death.

Non-physical methods of euthanasia utilizing inhalant agents (e.g. CO₂, inhalant anesthetics) should be followed-up with a secondary technique (e.g. decapitation) in order to ensure death, as all inhalant agents ultimately induce death via hypoxia.

Acceptable procedures for euthanasia of neonatal mice and rats less than 10 days old:

i. Decapitation

1. Decapitation, preceded by loss of consciousness via CO₂ or inhalant anesthetic administration, or hypothermia (see also SOP# 101 Rodent Anesthesia)
2. Overdose of injectable barbiturate IP

SOP#: 301.01

Page: 5 of 5

For euthanasia of mice and rats greater than 10 days old follow guidelines for adults.

For euthanasia of precocial rodent neonates (e.g. Guinea pigs) follow guidelines for adults.

Note: Near-term fetuses should be confirmed non-viable by monitoring for inactivity prior to disposal or processing (e.g. tissue collection/fixation). Follow guidelines for euthanasia of neonates.

4. PERSONNEL SAFETY

- a. Medical emergencies: call NUH 6779 5555.
- b. When working with animals wear appropriate PPE, observe proper hygiene, and be aware of allergy, zoonosis, and injury risks. Refer to the OSHE webpage for more information.

5. ANIMAL RELATED CONTINGENCIES

- Post contact information for emergency assistance in a conspicuous location within the animal facility.
- Emergency veterinary care is available at all times including after working hours and on weekends and holidays: **90013073**

6. REFERENCES

- Cornell University ACUP
<http://www.research.cornell.edu/care/documents/ACUPs/ACUP301.pdf>
- AVMA Guidelines on Euthanasia June, 2007
http://www.avma.org/issues/animal_welfare/euthanasia.pdf
- NIH Guidelines http://oacu.od.nih.gov/ARAC/documents/Rodent_Euthanasia_Pup.pdf
- http://oacu.od.nih.gov/ARAC/documents/Rodent_Euthanasia_Adult.pdf
- NACLAR Guidelines: http://www.ava.gov.sg/NR/rdonlyres/C64255C0-3933-4EBC-B869-84621A9BF682/13557/Attach3_AnimalsforScientificPurposes.PDF pg36.
- Guide for the Care and Use of Laboratory Animals 8th edition pp. 123-124
- NUS IACUC
<http://www.nus.edu.sg/iacuc/policies%20and%20guidelines/Euthanasia%20of%20Animals%20used%20for%20Scientific%20Purposes-2.pdf>

Revision #	Author	IACUC Approval/ Effective Date	SOP #:
.01	Anna Acuna	17 September 2012	301.01