



TITLE:
Rodent Euthanasia in NINDS Managed Animal Facilities

PURPOSE:

The purpose of this SOP is to establish acceptable methods for euthanasia of rodents housed in NINDS/AHCS managed animal facilities and to ensure that such methods are in accordance with existing Animal Research Advisory Committee (ARAC) and NINDS/NIDCD ACUC policies and guidelines.

GENERAL INFORMATION:

Euthanasia is the act of rapidly inducing humane death in an animal, with minimum pain, fear or distress. Euthanasia techniques should result in prompt unconsciousness followed by cardiac/respiratory arrest and ultimate loss of brain function. In addition, the technique should minimize any stress and anxiety experienced by the animal before unconsciousness.

The AHCS will euthanize culled research animals upon written request from the investigator. Investigators are ultimately responsible for euthanasia of animals in accordance with methods as stated in their protocol. Investigators may euthanize rodents in their lab, in the Building 35 SAF procedure rooms or in the Building 10 (ACRF) procedure room. Animals must not be euthanized in animal holding rooms or in the presence of other animals not being euthanized (including those waiting to be euthanized).

PROCEDURES:

- A. To request that the AHCS euthanize animals, investigative personnel must place an orange "Please EUTHANIZE" sticker, initialed and dated, on the cage card to identify cages of rodents they want the AHCS staff to euthanize. The entire cage will be euthanized. It is critical that the barcode is not covered with the euthanasia sticker.
1. The individual requesting euthanasia MUST initial and date the sticker. NO EXCEPTIONS. Stickers are available in each animal holding room. Using your hand put even pressure on the label to ensure it adheres completely to the cage card.
 2. Cages should be left on the rack in the original location. Cages are removed by the individual performing euthanasia. Animal care staff will not be responsible for euthanasia of individually identified animals, unless they are singly housed.
 3. Handwritten notes on cages are NEVER acceptable.
 4. Cages labeled for euthanasia MUST always have food and water.
 5. Cages labeled for euthanasia must maintain cage space requirements. Refer to [SOP 3200 Cage Space Requirements for Laboratory Rodents](#).
 6. Pre-weanling age pups <21 days old cannot be left without the mother.
 7. Euthanasia of animals by AHCS personnel is performed daily on weekdays. Routine euthanasia is not performed on the weekend/holiday by AHCS staff.
 8. Animals are to be euthanized within 24 hours after the sticker is placed on the cage; weekends/holidays are an exception.

9. When an orange "Please Euthanize" sticker is placed on a cage with an orange or pink "Watch" card, the Veterinary Technologist (VT) is responsible for checking for these cages when in the room and euthanizing as soon as possible.
- B. The Facility Veterinarian (vet) can authorize euthanasia of an animal for medical/humane purposes.
1. An attempt will be made to contact the appropriate investigator before the animal is euthanized. If contact is not possible, instructions as indicated on the Disposition Form (DF) are followed as closely as possible.
 2. The vet or VT notifies the investigator or his /her designee, per vet directive, via phone or e-mail as soon as possible.
- C. Fetuses and Pregnant Rodents.
1. Pregnant dams: the method chosen for euthanasia of a pregnant mother should ensure rapid cerebral anoxia to the fetus with minimal disturbance to the uterine environment minimizing fetal arousal. Recommended methods for euthanasia of the mother are CO₂ exposure with or without cervical dislocation. Death of the mother must be verified after euthanasia and prior to disposal. The vet should be consulted for considerations of other euthanasia agents.
 2. CO₂ alone for euthanasia of pregnant dams (E15-E21 days of pregnancy) and fetuses is only acceptable if the mother is kept under CO₂ for an extended time (>10 min).
 3. Fetuses 14 days or earlier in gestation: Neural development at this stage is minimal and pain perception is considered unlikely. Euthanasia of the mother or 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.
 4. Fetuses 15 days or later in gestation to birth: The neural development at this stage supports the likelihood that pain may be perceived. When removal of fetuses is necessary, decapitation with sharp surgical scissors with a minimum 4cm blade is acceptable physical methods of euthanasia (Refer to #1 above for euthanasia in utero).
- D. Euthanasia of Rodent Neonates.
1. Maturation of the nervous system occurs during the period just prior to birth and into the second week of postnatal life. Resistance to hypoxia at this age results in a prolonged time to unconsciousness when CO₂ is used as a euthanasia agent. A secondary physical method of euthanasia is recommended to ensure death (e.g. decapitation). Death must be verified after euthanasia and prior to disposal.
 2. Mouse and rat neonates up to 10 days of age:
 - i. AHCS staff standard procedure is CO₂ euthanasia until breathing has ceased (5-10 minutes) followed by decapitation.
 - ii. Acceptable methods for euthanasia include injection of chemical anesthetics (e.g., pentobarbital) or decapitation with sharp surgical scissors with a minimum 4cm blade.
 - iii. Anesthesia may be induced by inhalant or injectable anesthetics under an approved ASP. The facility vet should be consulted for appropriate agents and dosages.
 3. Neonates older than 10 days of age: Follow guidelines for euthanasia of adults as described below.
- E. Euthanasia of Adult Rodents.
1. CO₂ Asphyxia:
 - i. Must be performed in a manner that permits inflow in the chamber to be regulated (CO₂ tank with regulator or built-in/building system, providing there

- is a regulator). Experienced or trained personnel perform this procedure in a CO2 chamber, the home cage or other suitable container.
- ii. The number of animals in a cage during euthanasia must not exceed the number of animals that can be housed in the cage according to the Guide. Refer to [SOP 3200 Cage Space Requirements for Laboratory Rodents](#).
 - iii. Euthanasia in the home cage is always preferred.
 - iv. Only animals for immediate euthanasia should be present in the room.
2. Cervical Dislocation (without anesthesia):
 - i. As with all methods of euthanasia, cervical dislocation without anesthesia must only be performed by properly trained personnel with demonstrated high degree of technical proficiency, and as such is generally only performed by investigative staff under an approved ASP.
 - ii. Cervical dislocation without anesthesia is a conditionally acceptable method of euthanasia rats weighing < 200 grams and for adult mice.
 - iii. Cervical dislocation without anesthesia should be used only when scientifically justified by the user and approved by the PI's ACUC or by vet approval.
 3. Decapitation (without anesthesia):
 - i. This method of euthanasia is rapid and painless when performed by skilled personnel with well-maintained equipment. Therefore, only properly trained, experienced personnel will perform this procedure.
 - ii. Decapitation without anesthesia is conditionally acceptable if performed correctly. It must be justified by the user and approved by the PI's ACUC. This technique is generally only performed by investigative staff under an approved ASP. Acceptable methods of decapitation include use of sharp surgical scissors with a minimum 4cc blade capable of swift decapitation with one stroke, or a properly maintained guillotine.
 - iii. Refer to [SOP 6510-PI Guillotines in NINDS Managed Animal Facilities](#) for more information.
 4. Inhalant Anesthetic Agents (Isoflurane):
 - i. Isoflurane rapidly induces anesthesia and is an effective inhalant anesthetic. Isoflurane has low solubility and induces rapid anesthesia/euthanasia. However, it has a slightly pungent odor and animals may hold their breath, thereby, delaying the onset of action. Only trained personnel are allowed to use isoflurane for rodent euthanasia.
 - ii. Euthanasia should be ensured by performing cervical dislocation or bilateral pneumothorax.

REFERENCES:

1. AVMA Guidelines for the Euthanasia of Animals, 2013
<https://www.avma.org/KB/Policies/Documents/euthanasia.pdf>
2. ARAC Guidelines for the euthanasia of rodent feti and neonates,
http://oacu.od.nih.gov/ARAC/documents/Rodent_Euthanasia_Pup.pdf.
3. ARAC Guidelines for the euthanasia of rodents using carbon dioxide,
http://oacu.od.nih.gov/ARAC/documents/Rodent_Euthanasia_Adult.pdf.
4. *The Guide for the Care and Use of Laboratory Animals*, ILAR, NRC, 2011
5. [SOP 6510PI - Guillotines in NINDS Managed Animal Facilities](#)
6. [SOP 3200 Cage Space Requirements for Laboratory Rodents](#)

Attachments: None

Updates and/or Changes:

2/17/16:

- Changed title by removing NIDCD.
- Added in general information section that animals must not be euthanized in animal holding rooms or in the presence of other animals not being euthanized (including those waiting to be euthanized).
- Updated Guide and AVMA Euthanasia Guidelines references
- Hyperlinked SOPs

2/8/2019

- Removed cervical dislocation occurrences throughout the SOP.
- Added sharp surgical scissors with a minimum 4cc blade to describe the scissors used for decapitation.

4/9/19

- Updated Hyperlinks