



UNIVERSITY STANDARD

Title

UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL STANDARD ON RAT AND MOUSE EUTHANASIA

Introduction

PURPOSE

The standards and procedures described below provide guidance to all researchers and animal handlers for the euthanasia of rodents. Performing euthanasia correctly is an ethical imperative. Proper euthanasia minimizes pain/distress and reliably causes death.

SCOPE OF APPLICABILITY

All personnel engaged in the euthanasia of rodents. This includes Division of Comparative Medicine (DCM) personnel and trained research personnel, listed in the IACUC approved Animal Care Application (ACAP).

Note: In addition to DCM personnel, only research personnel who have been properly trained and are listed on the approved ACAP (IACUC protocol), can perform these techniques.

Note: In extenuating circumstances, and with permission from DCM Veterinary or IACUC staff members, an acceptable euthanasia method not described in the approved protocol may be performed in the animal housing rooms or elsewhere.

The UNC-CH IACUC expects that anyone involved in animal work at the University will comply with this Standard. Requests for exceptions to this Standard must be reviewed and approved by the IACUC.

Standard

All animals slated for euthanasia must be housed according to UNC-Chapel Hill cage density standards, and should have access to food and water if they are being housed for more than 3 hours prior to euthanasia. See links for: [Mouse Cage Density Standard](#) and [Rat Cage Density Standard](#).



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Unweaned animals that are slated for euthanasia should stay with the lactating female until final preparation(s) for euthanasia are complete.

Cages marked for euthanasia should not be overcrowded or stacked on top of each other, as this blocks air flow into the cage.

Euthanasia must follow the method(s) described in the approved ACAP.

Unintended recovery must be prevented by the use of appropriate CO₂ concentrations and exposure times, or by other means such as a secondary physical method. **A confirmation of death by a physical method is required for all animals, irrespective of age, unless otherwise approved in the protocol.** In cases where the approved protocol describes euthanasia utilizing automated CO₂ systems (such as the DCM provided Smartbox systems), personnel are not required to perform a secondary physical method.

The DCM staff can, for a fee, perform euthanasia of research animals. When requesting this DCM service, research personnel must do the following:

Complete and submit a “Request for Euthanasia of Animals” form [[available on the DCM website](#)]. Ensure all euthanasia instructions are very clear (e.g. ‘euthanize dam and neonates’ or ‘euthanize only the pre-weanling animals, not the dam’);

Leave the animal(s) requiring euthanasia in the cage. All unweaned animals should stay with the lactating female until the time of euthanasia.

Place a euthanasia card on the cage so that DCM can readily identify the animal(s) slated for euthanasia.

The investigator is responsible for ensuring proper documentation on a euthanasia request form. DCM is not responsible for errors on the form or miscommunications that may occur during the euthanasia process. Do not make verbal arrangements with DCM staff.

Euthanasia of sick or injured animals

Sick or injured animals that cannot be successfully treated or relieved of pain and distress should be euthanized promptly. Research personnel are responsible for euthanizing sick, injured or moribund animals as soon as these conditions are noted. These animals should not be held for later euthanasia by DCM personnel. To investigate unexpected illnesses, research personnel may contact Veterinary Services to arrange for euthanasia and necropsy of the animals. DCM veterinarians have the authority to euthanize moribund



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animals, as well as animals experiencing more than momentary or slight pain and/or distress. If the DCM veterinarian is unable to contact research personnel regarding the care or treatment of a moribund animal, DCM veterinarians or designated representatives are authorized to euthanize the animal. Ensure appropriate emergency contact numbers for all research personnel are posted in the animal facility.

Euthanasia of animals in animal housing areas

The [AVMA Guidelines for the Euthanasia of Animals: 2013 Edition](#) indicates: “Methods of euthanasia likely to elicit distress vocalizations or pheromones that other animals in the room could hear or smell may be best performed in another location, if transportation distress can be minimized.”

Euthanasia of rodents may take place in rodent housing rooms containing individually ventilated caging (IVC) and/or a fume hood that exhausts to the outside. The euthanasia method used should be described in the approved protocol.

MOUSE AND RAT EUTHANASIA

Section 1: Terms and Definitions

Secondary physical method to ensure death — in order to confirm that animals are dead, one of the following secondary physical methods must be performed on animals that have been anesthetized with approved agents: 1) cervical dislocation; 2) decapitation; 3) thoracotomy [open the chest cavity using sharp scissors or scalpel]; or 4) collection of vital organs.

Unanesthetized Physical Euthanasia — Individuals who perform physical euthanasia on unanesthetized animals must first be trained and certified by IACUC approved designees. Physical euthanasia on unanesthetized animals, irrespective of age, can only be done if the procedure is described in the approved ACAP.

- **Cervical Dislocation** — cervical dislocation in unanesthetized neonatal and adult rodents is permitted only if it is performed correctly by a trained person, and it is described in an approved ACAP. Manual cervical dislocation is a humane and acceptable method of euthanasia when limited to rodents weighing less than 200 grams. Personnel using cervical dislocation must be adequately trained, demonstrate their technical proficiency, and must consistently apply this method humanely and effectively.
- **Decapitation** — decapitation in unanesthetized neonatal and adult rodents is permitted only if it is performed correctly by a trained person, and it is scientifically



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justified in an approved ACAP. When performed properly this technique is nearly instantaneous and is considered humane. Guillotines that are designed to accomplish decapitation in adult rodents in a uniformly instantaneous manner are commercially available. Sharp scissors can be used to decapitate neonatal rodents. Check guillotine and scissor blades frequently to ensure sharpness. The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades. The use of plastic cones to restrain animals appears to minimize stress from handling, minimize the chance of injury to personnel, and improves positioning of the animal in the guillotine. ([AVMA Guidelines for the Euthanasia of Animals: 2013 Edition](#))

Note: The Physics Department's Instrument Shop, located in Phillips Hall 115A, will sharpen blades for a small fee (919) 962-1183.

Gaseous Carbon Dioxide (CO₂) - must be supplied using a compressed gas tank. The use of dry ice as a source of CO₂ for euthanasia is not permitted. (Refer to section 3A)

Inhalant Anesthesia- anesthetic agent(s) delivered as a volatile gas to the respiratory tract to induce anesthesia. Personnel should minimize their exposure to these agents as some are considered chemical hazards. These agents should only be used in a chemical fume hood, ducted biosafety cabinet or in a system with an active gas scavenging device. (Refer to section 3B below.)

Injectable Anesthesia- chemical agent(s) administered by injection with a needle and syringe to induce anesthesia. Common routes of injection include, but are not limited to, intraperitoneal (IP), intramuscular (IM) or intravenous (IV). Injectable anesthetics are easy to administer, require minimal equipment, and avoid safety concerns associated with inhalants. (Refer to section 3C below.)

Section 2: Euthanasia of Rodent Fetuses

Fetuses **up to 14 days** in gestation: Neural development at this stage is minimal and pain perception is considered unlikely. Euthanasia of the mother or removal of the fetus(es) should ensure rapid death of the fetus(es) at this stage of development.

Fetuses **15 days** in gestation to birth: The literature on the development of pain pathways suggests the possibility of pain perception at this point in gestation. Whereas fetuses at this age are not sensitive to inhalant anesthetics, anesthesia may be induced by injection of the fetus with a chemical anesthetic, or by deep anesthesia of the mother with a chemical agent that crosses the placenta, e.g., pentobarbital. Decapitation with sharp scissors and cervical dislocation are acceptable physical methods of euthanasia when performed by a trained person. The specific technique(s) employed must be described in



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the approved ACAP. When chemical fixation of the whole fetus is required, fetuses should be anesthetized prior to immersion in or perfusion with fixative solutions. Consult with one of the DCM veterinarians to learn more about fetal sensitivity to specific anesthetic agents. When fetuses are not required for study, the method chosen for euthanasia of a pregnant mother must ensure rapid death of the fetus.

Section 3: Procedures

A. **Gaseous Carbon Dioxide (CO₂)**: The [AVMA Guidelines for the Euthanasia of Animals: 2013 Edition](#) recommends that the gradual displacement rate of CO₂ into the euthanasia chamber should be 10-30% to minimize pain and distress. All calculations described below are for a DCM shoe box style rat cage at 30% displacement.

Note: DCM procedure rooms have dedicated CO₂ euthanasia chambers equipped with acceptable flow meters. Investigators who wish to perform CO₂ euthanasia outside of DCM facilities must adhere to all of the following principals and must purchase the same equipment utilized by DCM. Appropriate flow meters may be purchased from VWR and can be found through the UNC purchasing system, E-Pro, or at the following website: <https://us.vwr.com> (part number: 89012-426). To purchase appropriately sized euthanasia chambers, contact DCM at (919-843-7992).

1. Remove each animal from the housing chamber and place into the euthanasia chamber. Never place the housing chamber into the euthanasia chamber. Never pre-charge the chamber. Do not place different animal species in the chamber at the same time. Do not overcrowd the chamber. Each animal should have enough floor space available to lie down.
2. Place the stainless steel lid over the plastic cage. The lid should be connected to a CO₂ tank via a plastic hose.
 - a) Make sure the two holes on the top of the lid are not blocked, as these holes allow air to be pushed out by the heavier CO₂.
 - b) Make sure the plastic cage does not have an automatic watering opening.
3. Turn on the valve located on top of the CO₂ tank. Next, set the flow meter by adjusting the regulator valve on the left side of the flow meter (see photo on the next page):
 - a) Standard DCM Shoebox style RAT cage: 7.8 liters per minute (lpm)
 - b) Standard DCM Shoebox style MOUSE cage: 1.8 liters per minute (lpm)
 - c) Other CO₂ Chambers: Use the following formula to calculate the appropriate flow rate: $\frac{\text{Height} \times \text{width} \times \text{length}}{60} = \text{liters} \times .20 = \text{flow rate/minute (units = cm)}$



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4. Continue to allow CO₂ to flow into the chamber for one minute after breathing stops (approximately 6 minutes for mice and 8 minutes for rats). Young animals, certain strains of mice, and sick animals may require more time to become deeply anesthetized.
5. Once animals are fully anesthetized, immediately perform a physical method of euthanasia (i.e. cervical dislocation, thoracotomy, major organ harvest, or decapitation) to confirm death.
6. Note: If a terminal procedure (i.e. cardiac puncture, tissue collection) must be performed before the secondary physical method, ensure that animals remain deeply anesthetized and that a physical method of euthanasia is performed following the terminal procedure.
7. Place dead animals into a non-PVC containing bag. DCM provides these bags in a variety of sizes. Label the bag with the IACUC protocol #. Seal the bag securely. Place the bag with dead animal(s) into the DCM carcass freezer available in each animal facility. Please see the Policy on Rodent Carcass Disposal for more information.
8. Disinfect the euthanasia chamber bottom after each use.





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B. Inhalant Anesthetics (e.g. Isoflurane)

Induction chambers for inhalational anesthetics must allow animals appropriate floor space without being too large. Chambers that are too large require increased volumes of the anesthetic agent and may result in slow induction time. Where applicable, induction chambers must prevent animals from coming into direct contact with an anesthetic soaked material. The lid should fit snugly and the chamber must be used in a fume hood, a ducted biosafety cabinet, or with a properly functioning active scavenging system.

1. Pre-charge the anesthetic chamber by opening the vaporizer or placing two to three pieces of absorbent material on the bottom of the chamber. Add approximately 3-5 mls of isoflurane liquid to the absorbent material (amount of isoflurane is determined by the size of the chamber). Close the lid and wait 5 minutes for the liquid to form a volatile gas within the chamber.
2. Remove the lid of the chamber, quickly place the animals in the chamber, ensure the absorbent material is not in direct contact with the animal, and immediately close the lid.
3. The animals should become anesthetized in 2-5 minutes. Neonates require a longer period of time to anesthetize and should remain in the chamber for at least five (5) minutes.
4. When animals are completely recumbent and obviously deeply anesthetized, remove them from the chamber.
5. **Immediately** perform a physical method of euthanasia. Isoflurane is highly volatile and animals will quickly regain consciousness once removed from the chamber. Therefore, it is imperative that physical euthanasia be performed immediately.
6. Note: If a terminal procedure (i.e. cardiac puncture, tissue collection) must be performed before the secondary physical method, ensure that animals remain deeply anesthetized and that a physical method of euthanasia is performed following the terminal procedure.

C. Injectable Anesthetics

Injectable anesthetics can be effectively used to anesthetize animals prior to performing physical euthanasia. The agent should be an anesthetic recommended for



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the species, and the dosage used should be equal to or greater than the standard published reference dose for anesthesia (e.g., a common dose of pentobarbital for euthanasia is 100 mg/kg, which is approximately twice the anesthetic dose for rats and mice). Once the injectable anesthetic is administered, allow sufficient time for the animal to lose consciousness.

Injectable anesthetics intended for use in adult rodents may not have the desired effect in neonates. In a pilot study conducted at UNC-Chapel Hill, few anesthetics were found to be reliably effective in neonates. The drugs that provided the most effective anesthesia are available only to veterinarians and as a result were considered impractical for use by the scientific community. Contact a DCM veterinarian for more information about appropriate doses of injectable anesthetics.

D. Automatic Carbon Dioxide (CO₂) Systems

The automated euthanasia system includes three stages, starting with the push of a button: charge, dwell, and exhaust. Automation also eliminates the need for physical verification of death and its emotional impact on personnel. All individuals who operate this equipment must first review this document and receive hands-on training and certification. Completion of training must be documented.

Precautions:

- Red “Emergency Stop” button on the touch screen may be pressed at any time to stop the euthanasia cycle. **Gas will immediately stop flowing. Only use if the CO₂ hasn’t come into contact with the animals yet, in order to avoid unnecessary distress.**
- The chamber door cannot be opened while the system is operating.
- In the event of loss of power to the system, the system will immediately begin the evacuation process of CO₂.
- Report any issues you encounter while operating the machine to the DCM Facility Manager/Supervisor **IMMEDIATELY**.

Operation of Unit: The system works with preset timings that assure humane and efficient levels of CO₂. Gas flow rate is set at the source (fixed flow), while the timings are controlled by the automated controller unit.

1. If the system is off, press the “On” switch on the back of the automatic CO₂ system.
2. Turn the three pressure lock knobs on the front of the chamber door counterclockwise to open. The knobs must be in a fully horizontal position in order to open.



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3. Load the chamber with cages. The animal numbers within the cages must be in compliance with the applicable cage density policy.

- ❖ **Euthanizing animals contained in a single layer of cages:** Individual cage lids (filter tops) should remain on the cages, but must be unlatched (front and rear clasps) to break the seal and allow for the CO₂ to better enter the cage. Cages can then be loaded into the chamber.
- ❖ **Euthanizing animals contained in more than a single layer of cages (only in GMB UB):** A shelf divider is in place inside each of 3 chambers, so removal of filter tops and wire bar lids is necessary in order for the cages to fit inside the chambers.
 - Remove filter tops and wire bar lids from the first layer of cages and place under the divider panel in the chamber.
 - Remove filter tops and wire bar lids from the second layer of cages and place in the chamber on top of the divider panel.
 - Total number of cages accommodated per use will vary depending on actual cage size.

4. After loading the chamber, close the door and turn all three pressure lock knobs (in numerical order) fully clockwise to seal. If the knobs are not fully turned the system will not operate.

5. Engage the touch screen by tapping it with your finger. Then, press “START”.

6. Type in your assigned password and press “Next”.

7. Select the proper cycle to run depending on which species and the age of the animal you will be euthanizing.

8. Press “START” again. A picture of the chamber will appear on the touch screen and will flash green to indicate that the cycle has started and a countdown will begin.

9. The system will cycle through three stages:

- Charge: Gas flows through the chamber, fully charging the chamber with CO₂.
- Dwell: Gas flow stops and the chamber remains fully charged with CO₂.
- Exhaust: Exhaust blower turns on and purges the chamber, fully evacuating CO₂.

10. After the blower automatically switches off, the pressure lock knobs will release and the animals may be safely removed from the chamber.



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11. Remove all cages from the chamber.
12. Place all animals in plastic bags labeled with the ACAP ID# prior to placing in carcass box designated for incineration.
 - In addition to labeling with ACAP #, indicate “Euthanex Smartbox” or abbreviate with “ESB” on plastic carcass bag to distinguish them from animals requiring a secondary physical method of euthanasia.
13. Wipe down chamber using the preferred DCM disinfectant available in the facility.
14. Fill out Euthanex Log with date/time of use, room # (where animals came from), protocol #, and # of cages euthanized.

EXCEPTIONS

Requests for exceptions to this Standard must be reviewed and approved by the IACUC.

Definitions

IACUC: Institutional Animal Care and Use Committee

DCM: Division of Comparative Medicine

University Standard: The minimum acceptable limits or rules used to achieve Policy implementation, enforceable by the IACUC.

CO₂: Carbon Dioxide

ACAP: Animal Care Application (online application system name)

ESB: Euthanex Smartbox

AVMA: American Veterinary Medicine Medical Association

Related Requirements

EXTERNAL REGULATIONS AND CONSEQUENCES

AVMA Guidelines for the Euthanasia of Animals: 2013 Edition

<https://www.avma.org/KB/Policies/Documents/euthanasia.pdf>

UNIVERSITY POLICIES, STANDARDS, AND PROCEDURES

For more detailed guidance, please refer to the [University Policy on the Care and Use of Vertebrate Animals for Research, Training and Teaching Purposes](#).



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Contact Information

Subject	Contact	Telephone	Email
Policy/Training	OACU/IACUC	919-966-5569	iacuc@med.unc.edu
Logistics	DCM	919-962-5335	
Veterinary	DCM	919-843-3407	

Important Dates

- Effective Date and title of Approver: June 28, 2002; UNC IACUC
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Approved by: UNC IACUC

Dr. Mitchell Picker
UNC IACUC Chair

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