INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

RAT AND MOUSE EUTHANASIA POLICY

Performing euthanasia correctly is an ethical imperative. Proper euthanasia minimizes pain/distress and reliably causes death. Practical issues such as degree of technical difficulty, time required to perform the procedure, readily available equipment/resources to perform the procedure, as well as aesthetics and human emotion must be considered. Standardized guidelines for humane euthanasia are detailed in the June 2013 American Veterinary Medical Association (AVMA) Guidelines on Euthanasia and available HERE.

In addition to Division of Laboratory Animal Medicine (DLAM) personnel, only trained research personnel, listed in the IACUC approved Animal Care Application (ACAP), may euthanize animals. 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. 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 DLAM provided Smartbox systems), personnel are not required to perform a secondary physical method.

The DLAM staff can, for a fee, perform euthanasia of research animals. When requesting this DLAM service, research personnel must do the following:
1. Complete and submit a “Request for Euthanasia in Animals” form [available on the DLAM website]. Ensure all euthanasia instructions are very clear (e.g. ‘euthanize dam and neonates’ or ‘euthanize only the pre-weanling animals, not the dam’);
2. Leave the animal(s) requiring euthanasia in the cage. All unweaned animals should stay with the lactating female until the time of euthanasia.
3. Place a euthanasia card on the cage so that DLAM can readily identify the animal(s) slated for euthanasia.

The investigator is responsible for ensuring proper documentation on a euthanasia request form. DLAM is not responsible for errors on the form or miscommunications that may occur during the euthanasia process. Do not make verbal arrangements with DLAM 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 DLAM personnel. To investigate unexpected illnesses, research personnel may contact Veterinary Services to arrange for euthanasia and necropsy of the animals.

DLAM veterinarians have the authority to euthanize moribund animals, as well as animals experiencing more than momentary or slight pain and/or distress. If the DLAM veterinarian is unable to contact research personnel regarding the care or treatment of a moribund animal, DLAM
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. Note: In extenuating circumstances and with permission from DLAM Veterinary or IACUC staff members, an acceptable euthanasia method not described in the approved protocol may be performed in the animal housing rooms.

**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.

**Note:** In addition to DLAM personnel, only research personnel who have been properly trained and are listed on the approved ACAP, can perform these physical methods.

**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 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 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. ([2013 AVMA Guidelines on Euthanasia](https://www.avma.org/?)

**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** (CO$_2$): must be supplied using a compressed gas tank. The use of dry ice as a source of CO$_2$ for euthanasia is not permitted. (Refer to section 2A below.)
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 2B 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 2C 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 should ensure rapid death of the fetus 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 used by a trained person. The specific technique(s) employed must be described in 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 institutional 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 2013 AVMA Guidelines on Euthanasia 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 DLAM shoe box style rat cage at 30% displacement.

Note: DLAM procedure rooms have dedicated CO2 euthanasia chambers equipped with acceptable flow meters. Investigators who wish to perform CO2 euthanasia outside of DLAM facilities must adhere to all of the following principals and must purchase the same equipment utilized by DLAM. Appropriate flow meters must 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 DLAM at (919-843-7992).

1. 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.

2. Remove each animal from the housing chamber and place into the euthanasia chamber. Never place the housing chamber into the euthanasia 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.
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 DLAM Shoebox style RAT cage: 8 liters per minute (lpm)
   b. Standard DLAM Shoebox style MOUSE cage: 1.8 liters per minute (lpm)
   c. Other CO₂ Chambers: Use the following formula to calculate the appropriate flow rate:

   \[
   \text{Height} \times \text{width} \times \text{length} = \text{liters} \times .20 = \text{flow rate/minute (units = cm)}
   \]

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. DLAM provides these bags in a variety of sizes. Label the bag with the ACAP ID#. Seal the bag securely. Place the bag with dead animal(s) into the DLAM 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 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 DLAM veterinarian for more information about appropriate doses of injectable anesthetics.

D. **Automatic Carbon Dioxide (CO2) Systems**

The automated three-stage euthanasia cycle 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.**
- 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 DLAM Facility Manager/Supervisor IMMEDIATELY.

**Operation of Unit:** The system works with preset timings that assure humane and efficient levels of CO$_2$. Gas flow rate is set at the source (fixed flow), while the timings are controlled by the automated controller unit.

1. Press the “On” switch on the back of the automatic CO$_2$ 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.
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) can remain on the cages and cages can be loaded into the chamber.
   - **Euthanizing animals contained in more than a single layer of cages (only in Genetic Medicine):** Filter tops should be removed and a shelf divider should be used.
     - Place shelf divider panel into the chamber.
     - Remove filter tops from the first layer of cages and place the cages under the divider panel in the chamber.
     - Wire bar lids should be removed from cages when using the divider panel to allow panel to sit flush on top of the cages.
     - 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 CO$_2$.
   - Dwell: Gas flow stops and the chamber remains fully charged with CO$_2$.
   - Exhaust: Exhaust blower turns on and purges the chamber, fully evacuating CO$_2$.
10. After the blower automatically switches off, the pressure lock knobs will release and the animals may be safely removed from the chamber.
11. Place all animals in plastic bags labeled with the ACAP ID# prior to placing in carcass box designated for incineration.
   - In addition to required carcass information, indicate “Euthanex Smartbox” or abbreviate with “ESB” on plastic carcass bag to distinguish them from animals requiring a secondary physical method of euthanasia.
12. Remove all empty cages from the chamber.
13. Wipe down chamber using the preferred DLAM disinfectant available in the facility.

**EUTHANASIA REFERENCES**


