



INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE **RAT AND MOUSE EUTHANASIA POLICY**

Performing euthanasia correctly is an ethical imperative. Proper euthanasia is quick, minimizes pain/distress and reliably causes death. Practical issues such as degree of technical difficulty, time required to perform the procedure and readily available equipment/resources to perform the procedure as well as aesthetics and human emotion must also be considered. Standardized guidelines for humane euthanasia have been detailed in the June 2007 AVMA Guidelines on Euthanasia: http://www.avma.org/issues/animal_welfare/euthanasia.pdf

Euthanasia of animals

Animals may be euthanized by trained research staff, or they can be labeled for euthanasia which will be performed, with a fee for service, by Division of Laboratory Animal Medicine (DLAM) staff. [However see below for sick or injured animals]. When requesting DLAM service, research personnel must do the following: 1) complete and submit a "Request for Euthanasia in Animals" form (available at <http://research.unc.edu/dlam/downloadhusforms.htm>) and leave the animal(s) in the cage, and 2) place a euthanasia card on the cage so that DLAM can readily identify the animal(s) slated for euthanasia. Animals should always have food and water. Unless there is proper documentation on a euthanasia request form, the investigator is responsible for any errors or miscommunications that may occur during the euthanasia process. Do not make verbal arrangements with DLAM staff.

Young animals that are still nursing must not be removed from the lactating female. Leave the dam with the young, place a euthanasia card on the cage, and fill out a request for euthanasia form stating that only the pre-weanling animals are to be euthanized. Alternatively properly euthanize the young yourself.

All animals slated for euthanasia must be housed according to housing standards of UNC-Chapel Hill and the *Guide for the Care and Use of Laboratory Animals*. Cages marked for euthanasia must not be overcrowded. Do not stack cages slated for euthanasia on top of each other. This blocks air flow into the cage. **All personnel performing euthanasia are responsible for making sure that euthanasia is confirmed.**

Humane euthanasia of sick or injured animals

Sick or injured animals may require humane euthanasia if successful treatments are unlikely or if pain and distress cannot be appropriately managed. Moribund animals must also be euthanized in a timely manner. When sick, injured or moribund animals require euthanasia, it must be performed immediately; animals cannot be held for later euthanasia by DLAM personnel.

DLAM veterinary staff has authority to euthanize moribund animals or animals experiencing more than momentary or slight pain or distress. If DLAM veterinary staff is unable to contact research personnel regarding the care or treatment of a moribund animal, DLAM is authorized to euthanize the animal.

MOUSE AND RAT EUTHANASIA

* Section 1: Terms and Definitions

The following terms and definitions detail the UNC-Chapel Hill methods for conducting humane euthanasia in rats and mice. Exceptions must be described in an animal care and use protocol and reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) on a case by case basis.

Terms and Definitions

Physical methods of euthanasia—to ensure animals are dead, one of the following procedures 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 may be performed if tissues are needed for experimental purposes. Note: these physical methods can only be performed by personnel who have been properly trained. Cervical dislocation can not be performed on rodents that weigh greater than 200 grams.

Physical euthanasia performed on unanesthetized animals requires that all individuals performing the procedure are observed and certified by approved IACUC or DLAM staff members. Please note that Laboratory Coordinators **MAY NOT** certify persons performing physical euthanasia on unanesthetized animals. Performing physical euthanasia on unanesthetized animals can only be done if the procedures are described in an approved animal care application (ACAP).

Cervical Dislocation—cervical dislocation in unanesthetized neonatal and adult rodents is permitted only if it is performed correctly by a trained person, its use is scientifically justified, and it is approved by the IACUC in the animal use application. Manual cervical dislocation is a humane method of euthanasia when limited to rodents weighing less than 200 g and when performed properly by a certified person with a high degree of technical competency. 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, its use is scientifically justified, and it is approved by the IACUC in the animal use application. 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.” ([2007 AVMA Guidelines on Euthanasia](#)) Note: The Physics Department’s Instrument Shop, located in Phillips Hall 115A, will sharpen blades for a small fee.

Gaseous Carbon Dioxide (CO₂)—used in rodent euthanasia: CO₂ must be supplied using a compressed gas tank. The use of dry ice as a source of CO₂ for euthanasia is not permitted.

Inhalant Anesthesia—anesthetic agents that are delivered as a volatile gas to the respiratory tract and when used properly, effectively induce anesthesia. Personnel should minimize their exposure to these agents as several can be harmful (potentially causing liver damage or birth defects if pregnant women are exposed). These agents should only be used in a fume hood, ducted biosafety cabinet or in a system with an active gas scavenging device.

Injectable Anesthesia—chemicals that induce anesthesia when injected with needle and syringe: common routes of injection include intraperitoneal (IP), intramuscular (IM) or intravenous (IV). Advantages include the following: easy to perform, minimal equipment, and avoids safety concerns associated with inhalants.

* Section 2: Procedures

A. Gaseous Carbon Dioxide

DLAM procedure rooms have designated CO₂ euthanasia chambers

1. Place the Euthanex 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. Turn on the valve located on top of the CO₂ tank and set the flow to 6 PSI by adjusting the regulator valve. Pre-charge the chamber for 30 seconds.
3. Remove each animal from the housing chamber and place into the pre-charged euthanasia chamber. **NEVER PLACE THE ANIMALS INTO THE PRE-CHARGED EUTHANASIA CHAMBER WHILE STILL IN THE HOUSING 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.
4. Continue to allow CO₂ to flow into the chamber until animals are fully anesthetized (approximately 3 minutes). **Young animals, knockout mice, and sick animals may take longer to become deeply anesthetized.**
5. To make certain animals are dead, immediately perform a physical method of euthanasia. Note: if performing tissue collection or thoracotomy animals must be deeply anesthetized prior to these procedures. Note: it may take longer to anesthetize sick animals.
6. Place dead animals into a non-PVC containing bag. **DLAM provides these bags in a variety of sizes.** Label the bag with the animal care application number (IACUC ID). Seal the bag securely. Place bag with dead animal(s) into the refuse box in the dead animal cooler or freezer available in each building.
7. Wash the chamber cage bottom after each use.

B. Inhalant Anesthetics (e.g. Isoflurane)

Induction chambers for inhalational anesthetics must be of appropriate size so as not to overcrowd animals but not so large as to require large volumes of the anesthetic agent which slows induction time. Induction chambers must have a perforated platform to place the animals on to prevent direct contact with anesthetic soaked gauze (or other absorbent material). The lid should be tight fitting 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 placing two to three pieces of gauze on the bottom of the chamber, below the perforated platform. Add approximately 5 ml of isoflurane liquid to the gauze. Close the lid and wait 5 minutes for the liquid to form a volatile gas within the

chamber. (Note: the amount of isoflurane to add depends on the size of the chamber, so the actual amount needed may vary.)

2. Remove the lid of the chamber and quickly place the animals onto the perforated platform within the chamber and immediately close the lid.
3. The animals should become anesthetized in 2-5 minutes. Neonates must remain in the chamber for at least five (5) minutes.
4. When animals are completely recumbent and are 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; thus it is imperative that physical euthanasia be performed immediately.

C. Injectable Anesthetics

Injectable anesthetics can be effectively used to anesthetize animals prior to performing physical euthanasia. The agent should be a standard drug for the species and the dosage used should be equal to or greater than 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 animals are properly injected (usually IP), allow sufficient time for them 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.

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 due to loss of blood supply and non-viability of fetuses 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 time. Whereas fetuses at this age are not sensitive to inhalant anesthetics, euthanasia may be induced by the skillful injection of chemical anesthetics. Decapitation with surgical scissors and cervical dislocation are acceptable physical methods of euthanasia when used by a trained person. 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 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. 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.

EUTHANASIA REFERENCES

American Veterinary Medical Association (2007) AVMA Guidelines on Euthanasia. http://www.avma.org/issues/animal_welfare/euthanasia.pdf.

Anden NE, Magnusson T & Stock G (1974) Effect of anesthetic agents on the synthesis and disappearance of brain dopamine normally and after haloperidol, KCL or axotomy. *Naunyn-Schmiedebers Archiv fur Pharm* 283(4), 409-418.

Bergstrom DA, Bromley SD & Walters JR (1984) Dopamine agonists increase pallidal unit activity: attenuation by agonist pretreatment and anesthesia. *Eur J Pharm* 100(1), 3-12.

Bhathena SJ (1992) Comparison of effects of decapitation and anesthesia on metabolic and hormonal parameters in Sprague-Dawley rats. *Life Sciences* 50(21), 1649-55.

Brown RE (1995) An Introduction to Neuroendocrinology. *Cambridge*.

Holson RR (1992) Euthanasia by decapitation evidence that this technique produces prompt, painless unconsciousness in laboratory rodents. *Neurotoxicology and Teratology* 14(4), 253-257.

Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council (1996) Guide for the Care and Use of Laboratory Animals. National Academy Press (65-66).

Malyapa RS, et al (1998) DNA damage in rat brain cells after in vivo exposure to 2450 MHz electromagnetic radiation and various methods of euthanasia. *Radiation Research* 149, 637-45.

Mantz J, Varlet C, Lecharny JB, Henzel D, Lenot P & Desmonts JM (1994) Effects of volatile anesthetics, thiopental, and ketamine on spontaneous and depolarization-evoked dopamine release from striatal synaptosomes in the rat. *Anesthesiology* 80(2), 352-363.

NIH (2002) Guidelines for the Euthanasia of Rodent Feti and Neonates.