Institutional Animal Care and Use Committee		UNTHSC
Title: Euthanasia Guidelines		
Document #: 006	**Version #:** 02	
Approved by IACUC Date: February 28, 2017		

A. BACKGROUND INFORMATION

a. According to 9 CFR part 1, §1.1, "*Euthanasia* means the humane destruction of an animal accomplished by a method that produces rapid unconsciousness and subsequent death without evidence of pain or distress, or a method that utilizes anesthesia produced by an agent that causes painless loss of consciousness and subsequent death."

b. In accordance with 9 CFR part 2, §2.31(d)(xi), "Methods of euthanasia used must be in accordance with the definition of the term set forth in 9 CFR part 1, §1.1 of this subchapter, unless a deviation is justified for scientific reasons in writing, by the investigator."

c. According to the *Guide* (pg.123), "Euthanasia is the act of humanely killing animals by methods that induce rapid unconsciousness and death without pain or distress."

d. Euthanasia techniques should be consistent with the AVMA Guidelines for euthanasia, unless the deviation from these guidelines is justified in the protocol for scientific or medical reasons.

B. RESPONSIBILITIES

a. It is the responsibility of all investigators, staff, students and animal care staff using animals in research or teaching at the University of North Texas Health Science Center to follow this procedure.

C. PROCEDURES

a. All laboratory animals must be euthanized in a timely manner to prevent/ alleviate animal suffering, either as described in the approved protocol according to experimental endpoints, or as soon as necessary if established criteria for humane endpoints are reached.

b. All IACUC approved protocols must include a description of the methods for euthanizing the animals, and how death will be confirmed, especially in animals receiving anesthetics or CO_2 prior to euthanasia.

c. Animals must be euthanized only by trained personnel using appropriate technique, equipment, and agents. This is necessary to ensure a painless death that satisfies research requirements.

d. Death should be induced as painlessly and quickly as possible.

e. In effort to reduce stress in animals, euthanasia should not be performed in the animal housing room.
f. The euthanasia method must be appropriate to the species, approved in the animal study proposal and conform to the most recent AVMA Guidelines on Euthanasia.
g. The use of inhalant agents for euthanasia must observe the conditions and precautions spelled out in the pertinent sections of AVMA Guidelines. Administration of inhalant overdose may result in deep depression of all life signs prior to death. It is possible that animals could revive from this state, which can be mistaken for death during a cursory examination.
h. Below is a list of common methods of euthanasia for laboratory animals.

i. **Injectable Agents**
    i. **Injectable barbiturate combinations:** Injectable barbiturates, such as pentobarbital, are often used in combination with local anesthetics and anticonvulsants. An adequate dose of barbiturate is the most important component in these combinations.
        1. Pentobarbital and formulations containing pentobarbital, are controlled substances, which are regulated by the Drug Enforcement Agency (DEA). A single lethal IP (poultry, birds, mice, rats, hamsters and guinea pigs) or IV (rabbits) administration of pentobarbital of ≥120 mg/kg is sufficient for euthanasia. Pentobarbital sodium generally comes in various pharmaceutical formulations, either as pentobarbital (e.g. Nembutal) or as a mixture labeled for euthanasia only (e.g. FatalPlus, Beuthanasia, etc.).
    ii. **Dissociative agent combinations:** Lethal doses of dissociative agents such as ketamine can be used.
        1. In rodents, ketamine should be used in combination with an α-adrenergic receptor agonist such as xylazine.
        2. Doses and volumes of drugs may vary, but at least 4-5 times the anesthetic dose should be used for euthanasia.
    iii. To ensure death, administration of an injectable overdose must be followed by a secondary method of euthanasia such as:
        1. Cervical dislocation in poultry, birds, mice, hamsters, rats (<200 g), and rabbits (<1 kg)
        2. Decapitation
        3. Exsanguination
        4. Exsanguination as part of perfusion
        5. Bilateral thoracotomy
        6. Removal of organs for procurement

j. **MS 222** (aquatic species): Available as tricaine methane sulfonate (TMS), MS 222 can be used for the euthanasia of amphibians, fish, and other aquatic species. Tricaine is a benzoic acid derivative and generally should be buffered with sodium bicarbonate. A 10 g/L stock solution can be made, and sodium bicarbonate added to saturation, resulting in

a pH between 7.0 and 7.5 for the solution. The stock solution should be stored in a dark brown bottle, and refrigerated or frozen if possible. The solution should be replaced monthly and any time a brown color is observed. For euthanasia, a concentration ≥250 mg/L is recommended:

i. Fish: Fish should be left in this solution for at least 10 minutes following cessation of opercular movement. Large fish may be removed from the water, a gill cover lifted, and a concentrated solution from a syringe flushed over the gills.
ii. Amphibians: Amphibians should be left in this solution for at least 10 minutes following cessation of movement. MS 222 may also be injected into lymph spaces and pleuroperitoneal cavities.
iii. Death must be assured by a second form of euthanasia by one of the following methods as secondary methods to ensure euthanasia of aquatics:
    1. Pithing
    2. Decapitation
    3. Removal of multiple organs for tissue procurement
    4. Exsanguination

k. **Inhalants**

i. **Carbon Dioxide:** Carbon dioxide (CO_2) inhalation is the most common method of euthanasia used for poultry, birds, mice, rats, guinea pigs and hamsters. CO_2 exposure using a gradual fill method is less likely to cause pain due to nociceptor activation by carbonic acid prior to onset of unconsciousness. CO_2 must be used as follows:
    1. The euthanasia chamber should allow ready visibility of the animals. Do not overcrowd the chamber. All animals in the chamber must be able to assume normal postural adjustments.
    2. Compressed CO_2 gas in cylinders is the only recommended source of carbon dioxide because gas inflow to the chamber can be precisely regulated.
    3. An optimal flow rate for CO_2 euthanasia systems should displace 10% to 30% of the chamber or cage volume/min. Placing conscious animals in a pre-filled chamber is not acceptable as this is distressful to the animals.
    4. Animals should be euthanized in their home cage. If their home cage cannot be used, chambers should be emptied and cleaned between uses.
    5. Animals should be left in the container until clinical death has been ensured (wait at least 1 minute after the last animal's last breath). Unintended recovery must be prevented by the use of appropriate CO_2 concentrations and exposure times or by other means as defined below.
    6. The use of dry ice for CO_2 euthanasia is not permitted. The use of dry ice is a potential source of injury or distress if permitted to directly contact the animal.

ii. **Inhalants** (anesthetics other than CO_2)

1. Inhaled agents may be useful in cases where physical restraint is difficult or impractical.
2. When used as a sole euthanasia agent delivered via vaporizer of anesthetic chamber (open-drop technique), animals may need to be exposed for prolonged time periods to ensure death.

iii. To ensure death, administration of carbon dioxide or an inhalant anesthetic overdose in poultry, birds, mice, rats, guinea pigs and hamsters must be followed by a secondary method of euthanasia such as:
   1. Cervical dislocation in poultry, birds, mice, rats (<200 g), and rabbits (<1 kg)
   2. Decapitation
   3. Exsanguination
   4. Exsanguination as part of perfusion
   5. Bilateral thoracotomy
   6. Lethal IP (poultry, birds, mice, rats, hamsters and guinea pigs) or IV (rabbits) administration of pentobarbital (≥120 mg/kg)

l. **Physical Methods**

i. **Cervical Dislocation of Conscious Animals:** This conditionally acceptable technique is used to euthanize poultry, other small birds, mice, and immature rats (<200 g). It requires neither special equipment nor transport of the animal and yields tissues uncontaminated by chemical agents. Data suggest that electrical activity in the brain persists for 13 seconds following cervical dislocation, and unlike decapitation, rapid exsanguination does not contribute to loss of consciousness. The following guidelines must be observed when performing cervical dislocation in conscious animals:
   1. Individuals performing this technique must have a demonstrated high degree of technical proficiency as determined by the IACUC (Veterinarian or designee will observe the procedure).
   2. In heavier rats (>200 g) the greater muscle mass in the cervical region makes manual cervical dislocation physically more difficult and shall not be done.
   3. It is the PI's responsibility to determine that all personnel have been trained to perform this technique, and to monitor that personnel consistently apply it humanely and effectively.

ii. **Decapitation of Conscious Animals:** This conditionally acceptable technique is used to euthanatize rodents in research settings. It provides a means to recover tissues and body fluids that are chemically uncontaminated. It also provides a means of obtaining anatomically undamaged brain tissue for study. Loss of cortical function following decapitation is rapid and occurs within 5 to 30 seconds as measured by a significant reduction in amplitude recordings of visual evoked responses and EEG changes. Although it has been demonstrated that

electrical activity in the brain persists for 13 to 14 seconds following decapitation, more recent studies and reports indicate that this activity does not infer the ability to perceive pain, and in fact conclude that loss of consciousness develops rapidly. The following guidelines must be observed when performing decapitation in conscious animals:

1. Individuals performing this technique must have a demonstrated high degree of technical proficiency as determined by the IACUC (Veterinarian or designee will observe the procedure).
2. The PI is responsible for confirming that equipment used to perform decapitation is maintained in good working order, kept clean and serviced on a regular basis to retain blade sharpness. The use of plastic cones to restrain animals reduces distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal for decapitation.
3. It is the PI's responsibility to determine that all personnel have been trained to perform this technique, and to monitor that personnel consistently apply it humanely and effectively. Personnel should be trained on anesthetized and/or dead animals to demonstrate proficiency.

iii. Animals subjected to physical methods of euthanasia should be anesthetized or tranquilized prior to euthanasia. Physical methods without prior anesthesia or tranquilization must be scientifically justified in the approved protocol.

m. **Euthanasia of Rodent Fetuses and Neonates:** The following guidelines are based on recommendations by the NIH and are for the use of rodent fetuses and neonates:

i. Fetuses up to 14 days in gestation: Neural development at this stage is minimal and pain perception is considered unlikely. Rodent fetuses are unconscious in utero and hypoxia does not evoke a response. It is unnecessary to remove fetuses for euthanasia after the dam is euthanized.

ii. 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 less sensitive to inhalant anesthetics, euthanasia may be induced by the skillful injection of chemical anesthetics. Decapitations with surgical scissors or cervical dislocation are acceptable physical methods of euthanasia. When chemical fixation or rapid freezing (immersion in liquid nitrogen) of the whole fetus is required, fetuses should be anesthetized prior to immersion in or perfusion with fixative solutions. Anesthesia may be induced by hypothermia of the fetus, 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. The university veterinarian should be consulted for considerations of 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.

iii. Neonates up to 7 days of age: Acceptable methods for the euthanasia of neonatal mice and rats include: injection of chemical anesthetics (e.g., pentobarbital),

decapitation, or cervical dislocation. Additionally, inhalant anesthetics (e.g., isoflurane used with appropriate safety considerations), may be used. However, neonates have a high tolerance for hypoxia, so exposure must be prolonged (>20 minutes) and death confirmed by a secondary means. Pups should be anesthetized prior to freezing with liquid nitrogen. Similarly, anesthesia should precede immersion or perfusion with chemical fixatives. Anesthesia may be induced by inhalant or injectable anesthetics; the Veterinarian should be consulted for appropriate agents and dosages. Alternatively, when adequately justified, hypothermia for anesthesia may be used to induce anesthesia in pups younger than six days. When using hypothermia, there must be a barrier (petri dish, plastic wrap, etc.) between the pup and the ice.

iv. Neonates older than 10 days: Follow guidelines for adults.

n. **General Euthanasia Information**

i. A small fee will be charged to investigators who use the CO_2 tanks in the DLAM facilities. All staff who euthanizes animals using the DLAM CO_2 tanks must sign the log book in the room at the time of euthanasia.

ii. When a study is done and the animals or tissues are not needed, a request can be submitted for DLAM staff to euthanize the animals for the lab. A green euthanasia form will need to be completed (found at the West entrance to the vivarium) and an "X" placed on each cage card of each cage of animals to be euthanized. DLAM will euthanize the animals for a small fee.

iii. When euthanasia is performed in the lab, carcasses must be brought to DLAM staff for proper disposal. Refer to IACUC Document 016 Carcass Disposal.

iv. If animals are euthanized in the animal facility, lab staff must find a member of DLAM to give the carcasses to for proper disposal, as per IACUC Document 016 Carcass Disposal.

D. References:

a. 2013 AMVA Guidelines for Euthanasia of Animals: 2013 Edition
b. IACUC Document 016 Carcass Disposal
c. 9 CFR Animal Welfare Act
d. Guide for the Care and Use of Laboratory Animals, Eighth Edition