IACUC GUIDELINE: Euthanasia of Laboratory Animals

ACUP # 301  IACUC Approval Date: February 17, 2016

Purpose:
This Standard Operating Procedure (SOP) describes acceptable procedures for euthanasia of laboratory animals housed in the TAMU-C Animal Care Facility. It ensures that animals are euthanized in the most humane way possible.

Scope:
This guideline applies to all IACUC approved protocols subject to oversight by Texas A&M University-Commerce.

Materials:
• CO₂ euthanasia station
• Anesthesia or commercial euthanasia solutions
• Rodent guillotine

Responsibilities:
Appropriately trained staff members are expected to monitor laboratory animal health in accordance with this guideline. Animal care staff will conduct routine health surveillance and any concerns will be reported to the Attending Veterinarian immediately.

Procedures:
1) Euthanasia Methods Described
   a) CO₂ asphyxiation – Preferred method for rodents
   b) Barbiturate overdose – Preferred method for reptiles
   c) Overdose of inhalant anesthetic
   d) Tricaine methanesulfonate (TMS/MS222) – Preferred method for aquatic amphibians
   e) Physical methods

2) Euthanasia of Adult Rodents
   a) CO₂ asphyxiation:
      i) In order to minimize stress animals should be euthanized in their home cage, or a similar container with bedding and species-appropriate “distractors” (enrichment devices, food, etc.) to encourage normal exploratory behavior (do not pool mice of different sexes or aggressive males).
ii) Note: Neonatal animals (up to 10 days of age) are resistant to the effects of CO₂, therefore, alternative methods are recommended. CO₂ may be used for narcosis of neonatal animals provided it is followed by another method of euthanasia (e.g. decapitation using sharp blades). Keeping neonates warm during CO₂ exposure may decrease the time to death.

iii) Take container to lab with CO₂ euthanasia station (STC 256).

iv) Place container in fume hood adjacent to CO₂ tank.

v) Feed the CO₂ tank hose into the rodent container and cover (Do not pre-charge the chamber).

vi) Open the CO₂ tank valve and check the gauge. Adjust the flow rate to 2 to 3 L/min or 15psi. (More than 3 L/min or 15psi can cause undue distress and pain).

vii) Please note that the time required for euthanasia can be several minutes.

viii) Maintain the CO₂ flow until the animals have stopped breathing.

ix) Move the flow to zero and close the valve on the tank (clockwise turn).

x) Leave the animals in contact with CO₂ for an additional 2 minutes, minimum.

xi) To confirm death, monitor animal for the following signs: no rising and falling of chest, no palpable heart beat, poor mucous membrane color, no response to toe pinch, color change or opacity in eyes, release of feces and urine. Repeat as needed.

xii) Secondary physical method mandatory after CO₂. Bilateral thoracotomy must be performed on Rats >200g.

b) Barbiturate or injectable anesthetic overdose:
   i) Inject three times the anesthetic dose intravenously or intraperitoneally.
   ii) Animals should be placed in cages in a quiet area to minimize excitement and trauma until euthanasia is complete.
   iii) To confirm death, monitor animal for the following signs: no rising and falling of chest, no palpable heart beat, poor mucous membrane color, no response to toe pinch, color change or opacity in eyes.
   iv) A physical method of euthanasia, such as cervical dislocation, is recommended on your animals before disposal to ensure that they have been correctly euthanized.

c) Physical methods:
   i) Personnel performing physical methods of euthanasia must be well trained and monitored for each type of physical technique performed.
   ii) Anesthesia or sedation is necessary prior to physical methods of euthanasia, unless described in the Animal Use Protocol (AUP) and approved by the IACUC.
   iii) Cervical dislocation:
      1) For mice and rats, the thumb and index finger are placed on either side of the neck at the base of the skull or, alternatively, a rod is pressed at the base of the skull. With the other hand, the base of the tail or the hind limbs are quickly pulled, causing separation of the cervical vertebrae from the skull.
   iv) Decapitation:
      1) Guillotines that are designed to accomplish decapitation in adult rodents in a uniformly instantaneous manner are commercially available.
(2) The use of plastic cones to restrain animals is recommended as it reduces distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal in the guillotine.

(3) Guillotines are not commercially available for neonatal rodents, but sharp blades (e.g. scissors) can be used for this purpose.

(4) The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades.

v) Exsanguination:
   (1) Animals may be exsanguinated to obtain blood products, but only when they are deeply anesthetized.
   (2) Deeply anesthetize animal according to anesthetic directions.
   (3) Collect blood from the heart.
   (4) To confirm death, monitor animal for the following signs: no rising and falling of chest, no palpable heart beat, poor mucous membrane color, no response to toe pinch, color change in eyes.
   (5) A physical method of euthanasia, such as cervical dislocation or pneumothorax (the diaphragm is lacerated or the heart is removed to ensure death), is recommended on your animals before disposal to ensure that they have been correctly euthanized.

3) Euthanasia of Neonatal Rodents
   a) Euthanize rodents under 7 to 10 days old by one of the following procedures:
      i) CO₂ asphyxiation followed by decapitation.
      ii) Barbiturate overdose, IP.
      iii) Overdose of inhalant anesthetic followed by decapitation.
      iv) Decapitation (using sharp scissors).
   b) Rodents over 10 days old can be euthanized by the same procedures as adult rodents.

4) Euthanasia of Gestating Rodents
   a) Gestating rodents with fetuses under 17 days old can be euthanized by the same procedures as adult rodents.
   b) Gestating rodents with fetuses over 17 days:
      i) CO₂ asphyxiation of the mother, followed by decapitation or barbiturate overdose (IP) of the fetuses.
      ii) Overdose of injectable anesthetics to the mother.

5) Euthanasia of Ectothermic Vertebrates (amphibians, reptiles or fish)
   a) Non-physical methods:
      i) Injectable agent (sodium pentobarbital): Aquatic amphibians or fish
         (1) Inject sodium pentobarbital (intra-venously or intracoelomic) into the fish or amphibian at a dose of 60 to 100 mg/kg body weight.
         (2) Verify the animal is dead before disposing of the carcass by monitoring for respiratory/opercular movement (amphibian/fish respect) and lack of response.
to sharp tail pressure. Time to effect may vary, with death occurring in up to 30 minutes.

(3) If sodium pentobarbital does not produce death, follow the injection with a physical method of euthanasia to ensure death.

ii) Injectable agent: Reptiles or terrestrial amphibians.

(1) Adult reptiles are generally painlessly killed by use of a chemical anesthetic such as sodium pentobarbital via intracoelomic injection. The euthanasia agent T-61 (National Laboratories) is also effective on reptiles.

iii) External or topical agents: Aquatic amphibians or fish

(1) Tricaine methanesulfonate (TMS/MS222):

(a) MS222 is acidic and in concentrations >500 mg/L, it should be buffered with sodium bicarbonate to saturation resulting in a solution pH of 7.0 to 7.5.

(b) Tank method:

(i) Place animal in a solution of MS222 dissolved in water (minimum concentration of 250 mg/L) until death is achieved.

(ii) Verify the animal is dead before disposing of the carcass by monitoring absence of respiratory (amphibian) or opercular (fish) movement for at least 3 minutes.

(c) Alternative method.

(i) Remove animal from water and flush gills with a concentrated solution of MS222 (>250 mg/L).

(2) Benzocaine hydrochloride:

(a) Place animal into a bath of benzocaine hydrochloride solution of >250 mg/L.

(3) 2-phenoxyethanol:

(a) Place animal into a bath of 2-phenoxyethanol solution at a concentration of 0.5 to 0.6mL/L or 0.3 to 0.4mg/L.

iv) Inhalant methods cannot be used for many amphibians or reptiles due to their ability to hold their breath while surviving for long periods of anoxia. CO₂ asphyxiation can be used with more active species (most snakes and lizards)- note that while unconsciousness develops rapidly, time until death may be prolonged. A physical method of euthanasia (eg., pithing) is recommended to assure death.

b) Physical Methods:

i) Anesthesia or sedation must be applied prior to the use of physical techniques unless approved by the IACUC.

ii) Decapitation:

(1) Use sharp equipment of the appropriate size for the species to be euthanized to ensure that the head is quickly separated from the body rapidly and completely.

(2) Follow decapitation with pithing.

iii) Pithing:
(1) Insert a rigid metal rod into the foramen magnum which is identified by the slight midline skin depression posterior to the eyes when the neck is flexed. Ensure that both the brain and the proximal end of the spinal cord are destroyed.

(2) Follow pithing with decapitation.

iv) Thermal shock (freezing): Thermal shock can only be used for specimens that are <5 cm in total body length.

(1) Rapid freezing:
   (a) Euthanize small fish or amphibians instantly by immersion in liquid nitrogen.

(2) Slow freezing (thermal shock):
   (a) Option 1: Place fish in a container of system water and place in the freezer; as the temperature drops, the fish’s metabolism slows, and the fish will sink to the bottom of the container.
   (b) Option 2 (**From ZIRC): Set up an ice bucket or cooler with ice slush; form a depression in the ice to expose water; pour fish and larvae into the depression; use the minimal amount of water needed that will still prevent direct contact with ice.

6) Safety Practices for Personnel Using MS-222
   a) Wear protective clothing, gloves and goggles when handling the MS-222 powder.
   b) If possible, work inside a fume hood to prepare a concentrated stock solution by mixing an appropriate amount of MS-222 powder in a small volume of water. Inhalation of the powdered solution should be avoided.
   c) Dilute the stock solution further as required.
   d) Wear gloves and use a utensil to stir until all powder is dissolved.
   e) Wear gloves to handle animals exposed to MS-222.
   f) Dispose of MS-222 wastes by flushing down the drain to a sanitary sewer with an excess of water. MS222 wastewater should be diluted and flushed down the drain into a sanitary sewer system, never to surface water.
   g) If in a remote location where a sewer may not be readily available, further dilute the solution with water and dump wastes on land, in a location away from water.
   h) Do not discard MS-222 directly into surface water, storm water conveyances or catch basins.

4. Personnel Safety
   • Medical emergencies: call 911
   • When working with animals wear appropriate PPE, observe proper hygiene, and be aware of allergy, zoonosis, and injury risks. Refer to the TAMUC Occupational Health and Safety webpage for more information.

5. Animal Related Contingencies
   a. Post contact information for emergency assistance in a conspicuous location within the animal facility.
   b. Emergency veterinary care is available at all times including after working hours and on weekends and holidays: AV Dr Taylor 214-532-3420
References:


History: Version 01
Warren Thompson, Petra Collyer