Procedure # IBT-205.02  IACUC Approval: October 23, 2017

**IACUC Procedure:** Euthanasia

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**Purpose:**
The purpose of this document is to define the procedure regarding appropriate euthanasia.

**Scope:**
This procedure applies to all animal care and use subject to oversight by the Texas A&M University Houston/Kingsville Institutional Animal Care and Use Committee (IACUC).

**Responsibilities:**
The IACUC must review and approve methods of euthanasia submitted by the Principal Investigator (PI).

The Attending Veterinarian (AV) answers questions or concerns about the methods of euthanasia described in this procedure.

All Researchers must utilize only the methods of euthanasia approved in their Animal Use Protocol (AUP).

**Definitions:**
AVMA – American Veterinary Medical Association  
Euthanasia – “Good Death” in Greek

**Procedure:**

**I. Categorization of Euthanasia (as outlined in the AVMA Guidelines on Euthanasia.)**

1. Acceptable-Consistently produce a humane death when used as the sole method of euthanasia.
2. Acceptable with conditions-Certain conditions must be met to produce a humane death, may have greater potential for operator error or safety hazard, are not well documented in the scientific literature, or may require a secondary method to ensure death.

**II. General considerations**

1. *In all cases,* the techniques used must be **reviewed and approved by** the IACUC prior to commencement.
2. When a method considered “acceptable with conditions” per the AVMA guidelines is proposed, scientific justification is required. This includes cervical dislocation and decapitation as the sole method of euthanasia on mice or rats over 10 days of age.
3. Euthanasia should be performed in procedure rooms or laboratory space away from other mice and rats, when possible.
4. Death must be ensured prior to bagging the animal for disposal.
5. Personnel performing euthanasia must be properly trained in their approved technique to ensure that pain and distress are minimized.
6. Chemical methods of euthanasia (CO₂ chamber, inhalant anesthetics) must be followed with a physical method (cervical dislocation, bilateral thoracotomy, or exsanguination) to ensure death unless otherwise justified.
7. Animals should be handled gently as possible prior to euthanasia, and an examination must be performed after euthanasia to confirm the absence of a heartbeat.
8. The heart can still beat after breathing has stopped, so simply checking for the cessation of respiration is not adequate.
When animals need to be euthanized by the PAR for humane/veterinary reasons (may or may not be on protocol), animals will be humanely euthanized as per PAR euthanasia procedure.

III. **Use of Carbon Dioxide in Euthanasia of Rodents**

A CO₂ chamber is the most commonly used method of euthanasia for small rodents such as mice, rats, hamsters and guinea pigs. CO₂ must originate from a compressed gas cylinder equipped with the appropriate regulator/flowmeter assembly. CO₂ generated from dry ice is not an acceptable method of euthanasia. The chamber must allow visualization of the mice and rats during euthanasia.

Proper technique must be followed to ensure a humane death, as CO₂ may have noxious properties. Place the mice or rats into a clean chamber that has not been pre-charged with CO₂ or utilize the rodent’s home cage. Do not overcrowd the chamber—all mice or rats must be able to make normal postural adjustments. Rodents from different cages should not be combined in the chamber as this increases distress. Gradually increase the flow of CO₂ into the sealed chamber to minimize distress. A displacement rate from 10%-30% of the chamber volume per minute is recommended. Leave the mice or rats in the chamber for 4-5 minutes until respiration has ceased. Remove the mice or rats and confirm the absence of respiration. A physical method (cervical dislocation, bilateral thoracotomy, and exsanguination) must be used in conjunction with CO₂ to ensure death.

Special Considerations for Rodent Fetuses & Neonates:

CO₂ is **not** an effective method of euthanasia for rodent fetuses & neonates less than 14 days of age. One of the following methods should be utilized:

1. **Fetuses**
   a. **Fetuses up to 14 days of gestation:** Euthanasia of the dam or removal of the fetus results in rapid fetal death since fetuses cannot survive outside of the uterus.
   b. **Fetuses from 15 days of gestation to birth:** Decapitation with surgical scissors or cervical dislocation are acceptable physical methods. An injection of a chemical anesthetic overdose is an acceptable chemical method.

2. **Neonates**
   a. **Neonates up to 5 days of age:** Hypothermia may be used as a method of anesthesia provided they are not placed directly on the frozen surface (i.e., place them in a latex bag or cloth).
   b. **Neonates up to 14 days of age:** Injection of chemical anesthetics, decapitation, and cervical dislocation are acceptable methods of euthanasia. Cervical dislocation or decapitation without anesthesia requires scientific justification, review and approval by the IACUC.
   c. **Neonates older than 14 days and precocial young should be treated as adults.**

IV. **Rabbits**

Barbiturates overdose is a commonly used method for euthanizing rabbits. If rabbits are used to handling, venous access may be obtained via the ear. In the case of fractious rabbits, sedation may be necessary to gain venous access for administration of an injectable barbiturate or injectable barbiturate combination. Barbiturates may also be administered IP.

   a. Chemical methods commonly employed include barbiturate overdose (such as Beuthanasia-D Special at 1cc per 10 lbs body weight, IV.) Intramuscular sedation may be necessary prior to venipuncture.
   b. For instances where a terminal procedure is being performed under anesthesia and typical venipuncture is not practical, surgical plane anesthesia is confirmed by the absence of ocular and pedal reflexes. Then the animal is euthanized by 1-2cc Beuthanasia-D Special IC. Asystole is confirmed with a stethoscope. To insure that the rabbit does not recover from the injection either decapitation or bilateral thoracotomies are performed.
   c. Rabbits may also be euthanized by exsanguination. This is commonly done to prepare tissues for histology. First the rabbit must be under deep general anesthesia confirmed by the absence of ocular
and pedal reflexes. Then the chest is opened. With the heart beating, a cut is made in the right atrium and saline injected slowly in the left ventricle until all the blood is removed from the body.

V. Frogs
A commonly used method for euthanizing frogs used in research is immersion using an overdose of buffered Tricaine-S (MS-222) solution. It is important to note that amphibian hearts can beat even after brain death, so a physical method (such as decapitation) should be used following overdose immersion to ensure death.

NOTE: The above listed methods of euthanasia are not in any way intended to be a comprehensive list, but are rather examples of common euthanasia methods for the listed species. See the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition and/or contact the Attending Veterinarian for additional guidance.

References:
5. ARENA/OLAW Institutional Animal Care and Use Committee Guidebook, 2nd Ed. 2002.

History:
Version 00 – Approval: January 27, 2014
Version 01 – Approval: September 26, 2016
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