Institutional Animal Care & Use Program - UTEP	
Title: Guidelines for Euthanasia	
Policy#: 008	**Date in Effect**: 21 November 2014
Version: C	**Rev Date**: 23 March 2026
In Effect ☒ **Rescinded** ☐	**Date Rescinded**:

A) Responsibilities

It is the responsibility of all personnel using animals at The University of Texas at El Paso (UTEP) to abide by this policy. Exceptions to this policy must be approved by the Institutional Animal Care and Committee (IACUC) in the protocol to deviate from this policy.

B) Application

This policy applies to applicable animals used in research and teaching at UTEP.

C) Background Information

To minimize animal suffering, laboratory animals must be euthanized either as described in the protocol at established endpoints, or expeditiously if criteria for humane endpoints have been reached. Animals must be continually observed and never be left unattended during the euthanasia procedure. All methods used must result in the confirmed death of the animal; for most methods this requires a secondary physical method after the primary chemical method to ensure death, such as cervical dislocation, removal of vital organs, decapitation, or bilateral thoracotomy, depending on the species. Secondary physical methods of euthanasia are also a means to confirm death in reptilian, amphibian, and aquatic species. Animal carcasses and tissues must be properly disposed of after euthanasia. Deceased animals must not be left in cages. ***Confirmation of death is required before disposing of carcasses.***

It is the PI's responsibility to determine that all personnel have been trained to perform the protocol-approved method of euthanasia. Personnel performing euthanasia must follow the approved procedures and apply them humanely and effectively. Training can be provided within the lab group if the existing staff has adequate expertise. Additional training in these techniques is available from LARC (Laboratory Animal Resource Center).

This policy is based on the 2020 AVMA Guidelines for the Euthanasia of Animals.

D) Rodent Euthanasia

Laboratory rodents should be euthanized in their home cage or a new cage and must **not** be placed or recombined in unfamiliar groups. Activities that contribute to distress in rodents include transport, handling (in animals not accustomed to it), disruption of compatible groups, and elimination of established scent marks. While eliminating all sources of distress may not be possible, the selected method of euthanizing rodents must minimize these sources of potential distress. Methods of euthanasia likely to elicit distress vocalizations or pheromones that other animals could hear or smell should be performed in a dedicated procedure or post-mortem room, if transportation distress can be minimized. Rodent fetuses are in a state of unconsciousness during pregnancy, so euthanasia of the dam is considered sufficient to euthanize fetuses if they remain in the uterus. However, if fetuses must be removed from the uterus for tissue collection or experimentation, they should be treated as altricial neonates.

1. Chemical Methods

a. Carbon Dioxide Inhalation (≥10 days old)

- CO_2 exposure using a gradual fill method with a displacement rate from 30% to 70% of the chamber volume/min is recommended; see Appendix A.
- CO_2 must be supplied in a precisely regulated and purified form without contaminants or adulterants, typically from a commercially supplied cylinder or tank. An appropriate pressure-reducing regulator and flow meter is absolutely necessary.
- CO_2 flow should be maintained for at least 1 minute after respiratory arrest.
- Rodents should be kept in their home cage with familiar cage mates during CO_2 administration.

- The practice of immersion, where conscious animals are placed directly into a container prefilled with 100% CO_2, is unacceptable.
- See table in Appendix B for exposure times and general information.
- Dry ice as a source of CO_2 is not acceptable under any circumstances.

b. Injectable Anesthetic Overdose
   - Intraperitoneal injection of at least 200 mg/kg sodium pentobarbital is recommended.
   - Sodium pentobarbital containing solutions can be viscous and are best diluted to a concentration of no more than 60 mg/ml.
   - Other injectable anesthetics may be approved and delivered at an overdose.
   - Tribromoethanol (Avertin) is acceptable with conditions as a method for euthanasia of laboratory rodents Its use requires scientific justification. Recommended dose of greater than 500 mg/kg IP.
   - All chemical methods should not be used as a sole agent and must accompany a physical method of euthanasia described in section H. of this policy

c. Inhalant Anesthetic Overdose
   - Isoflurane inhalation during an overdose may be utilized as a method of euthanasia, preferably by precision vaporizer. Concentration more than or equal to 5% and duration: at least one minute after breathing stops.
   - Open drop method is acceptable with a minimum of 5% *(see policy)*
   - POLICY: Isoflurane Waste Anesthetic Gas
   - Chambers must not be pre-charged with anesthetic and must be gradually brought to the 5% concentration.

d. Neonates (<10 days old)
   - Anesthetic overdose, as listed in the chemical methods above, can be used.
   - Decapitation using scissors or sharp blades is acceptable as a sole means of euthanasia for most protocols.

E. Zebrafish Euthanasia

Approved methods for zebrafish vary by age and agent.

a. Adults and Fry (>3 days post fertilization and older)
   - Immerse fish in a solution of pharmaceutical grade MS-222 (e.g., Syncaine). The solution must be buffered with sodium bicarbonate to a pH of 7.0-7.5.
   - Keep fish in an immersion euthanasia solution for at least 30 minutes after cessation of opercular movements when using MS-222. If animals are too young to observe opercular movement, keep immersed for 30 minutes.
   - A secondary method of euthanasia/confirmatory method (exsanguination, decapitation, etc.) is required, even after cessation of opercular movement is noted.

b. Other solutions inclusive of eugenol, isoeugenol and clove oil require fish to remain in the solution for at least 10 minutes after cessation of opercular movements. Other solutions require fish to remain in the solution for at least 30 minutes after cessation of opercular movements. If animals are too young to observe opercular movement, keep immersed for 30 minutes.

c. Rapid chilling
   - Submerge fish in 2-4°C water for 10 minutes for zebrafish >7 days post fertilization and older and 20 minutes for zebrafish 4-7 days post fertilization.

d. Sodium or calcium hypochlorite may be used as a single agent on zebrafish 4-7 days post fertilization. Animals should be immersed for at least 5 minutes.

Embryos (<3 days post fertilization)

e. Tricaine, other agents, or rapid chilling may be used as above, but embryos <3 days post fertilization should be followed with an adjunctive method.
   - Adjunctive methods include immersion in sodium (10%) or calcium hypochlorite (10%).

f. Sodium or calcium hypochlorite may be used as a single agent on zebrafish embryos <3 days post fertilization. Animals should be immersed for at least 5 minutes.

F. Reptile and Amphibian Euthanasia

a. Chemical Methods

- Pharmaceutical grade MS 222 (e.g., Syncaine) can be used either as an injectable agent (except intracoelomic injection in amphibians). Amphibians should be left in this solution for at least 1 hour following cessation of movement. Amphibians may also be fully anesthetized in a properly buffered TMS 222 bath with a minimum 4-minute immersion that results in cessation of movement prior to application of euthanasia via a physical method. Alternatively, a minimum of 1 hour of immersion is recommended to ensure reliable euthanasia, especially for larger amphibians.
- For reptiles, an intracoelomic injection of 250-500mg/kg of neutral pH solution (0.7% - 1.0% MS 222) typically results in complete loss of consciousness by 4 minutes post-injection; unbuffered, 50% TMS 222 must be administered via intracoelomic injection to complete euthanasia.

b. Injectable Anesthetic Overdose

Sodium pentobarbital (60 to 100 mg/kg of body weight) can be administered IV, intracoelomically, in the subcutaneous lymph spaces, or in the lymph sacs, although doses vary by species. Please consult with the Attending Veterinarian.

G. Use of High Caliber Firearms (Field Studies)

Due to lack of control over free-ranging wildlife and the stress associated with close human contact, use of a firearm may be the most appropriate means of euthanasia for field studies.

- Scientific justification is required and must be approved by the IACUC Committee.
- Use of a firearm must be appropriate for the species and circumstances.

- Firearms may only be used by trained and competent personnel with documentation provided in the attachments section of the protocol showing competence. Documentation may include but is not limited to valid state or country of study permits, Texas hunting license or multi-state license where the study will be performed, all required permits subject to forestry or other governing bodies, certificate of completion of an approved Hunter Education program, certificate of completion in firearms training, or other related certification program.
- The muzzle must never be pressed directly against the animal's head.
- Ensuring awareness of what is behind the target because bullets may exit the skull and travel beyond the animal.

The table below outlines information that should be included in the proposed method outlined in the IACUC protocol:

Category	What Must Be Specified
Firearm Type	Handgun, rifle, shotgun; gauge/caliber; justification for choice.
Ammunition Type	Bullet type (solid-point, hollow-point, slug, birdshot); expected penetration.
Caliber / Ballistics	Information confirming sufficient muzzle energy for species, size, skull thickness.
Anatomical Target	Proper placement landmarks ensuring instantaneous death (as defined by AVMA-aligned guidance).
Distance and Firing Method	Required stand-off distance, operator position, safety measures.
Species-appropriateness	Match firearm parameters to animal species and age.
Safety Considerations	Risk assessment for operator, bystanders, and surroundings.
Secondary Method (if required)	Confirmation that a follow-up method will be used when AVMA requires it.

H. Physical Means

a. Decapitation

- Decapitation is acceptable with conditions and only when required by experimental design and must be justified and approved on the IACUC protocol. Documented training for this procedure is required.
- Equipment used for decapitation must be maintained in good working order and serviced regularly to ensure sharpness of blades.
- Guillotines and scissors must be clean, rust-free, sharp, and move freely without resistance.
- Before each use, check for rust, damage, tension, cleanliness, and smoothness of operation.
- Blades must cut cleanly with minimal force and without dragging or binding.
- Guillotines must have documented regular sharpening at least annually or every 100 uses, whichever comes first.
- Scissors or razor blades must be new or newly sharpened, and dull devices must be sharpened or discarded in a rigid sharps container.

b. Sharpness testing is required before each use. Examples include:
   - Test on paper, sponge, carrot, rubber band, or polyethylene tubing.
   - The cut must be clean, quick, and smooth.
   - If it fails, the equipment must not be used until sharpened or repaired.

c. Cleaning and sanitization requirements
   - After each decapitation equipment must be rinsed or wiped down with an approved disinfectant to remove blood or tissue debris.
   - Documented quarterly cleaning is required including disassembly and disinfection of all surfaces using an approved disinfectant.
   - Documented lubrication of moving parts is also required during quarterly cleanings.

d. UTEP IACUC requires maintenance logs, including:
   - Dates of sharpening, lubrication, cleaning, and service
   - Sharpness test results
   - Number of animals euthanized

- Documentation must be available upon request for semiannual inspections.

e. Cervical Dislocation
    - Cervical dislocation is acceptable with conditions and must be justified and approved on the IACUC protocol. Documented training for this procedure is required.
    - The method should only be performed by individuals who have demonstrated the physical strength and technical proficiency to perform it quickly and humanely.

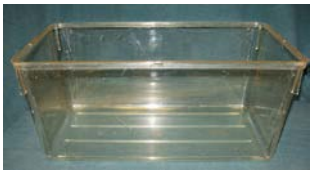
f. Bilateral Thoracotomy
    - Used as a confirmatory secondary method by opening the thoracic cavity to ensure cessation of cardiac function.

g. Exsanguination (rats)
    - Used as a secondary method under deep anesthesia. Removal of a sufficient volume of blood through the caudal vena cava to cause death.

h. Organ removal/ Perfusion
    - Used as a secondary method under deep anesthesia. Removal of the heart.
    - Perfusion – Used as a secondary method under deep anesthesia. The circulatory system is flushed with a solution (saline/fixative) by accessing the heart via the thoracic cavity causing rapid circulatory collapse.

Appendix A

Rodent Euthanasia CO_2 (30 - 70% Concentration) Recommended Flow Rates per Cage Type and Size

Cage Type	Image of Cage	Concentration of CO_2	Flow Rate Setting on CO_2 Flow Meter
Rat Tecniplast		30-70%	6.96 -16.24 L/min-
Rat Standard		30-70%	5.64 – 13.16 L/min
Mouse Tecniplast		30-70%	2.58 – 6.02 L/min

Appendix B

Age Group	Species	CO_2 Fill Rate Requirement	Approx. Time to Respiratory Arrest	Required Additional CO_2 Flow	Minimum Total Exposure Time	Notes
Adults	**Mice**	30–70% chamber volume per minute	Typically, ~4–6 minutes, depending on chamber volume and fill rate	At least 1 minute after respiratory arrest	Approximately 5–7 minutes total	Continuous observation required; gradual fill must be used
Adults	**Rats**	30–70% chamber volume per minute	Typically, ~4–6 minutes under proper fill conditions	At least 1 minute after respiratory arrest	Approximately 5–7 minutes total	Confirm death physiologically (no heartbeat or respiration)
Neonates (0–7 days)	**Mice & Rats**	Same CO_2 fill rate rules as adults (30–70% chamber volume per minute)	Much more resistant to CO_2; longer time required	Secondary physical method required (decapitation etc.)	Minimum of 10 minutes CO_2 exposure	CO_2 alone is unreliable in neonates; must use physical confirmatory method