



IACUC Guidance:	TAMU-G-048	Title:	Guidelines on Euthanasia of Fish, Amphibians and Reptiles	
	Location	Effective Date	Review By	
	College Station/Dallas/Galveston/Kingsville	10/20/2022	05/31/2025	
	Houston	N/A	N/A	

1. PURPOSE

- 1.1. To describe common methods of euthanasia of fish, amphibians and reptiles used for research, teaching, testing and other purposes at Texas A&M University.

2. SCOPE

- 2.1. Applies to commonly used methods of euthanasia. For a complete review, see the current AVMA Guidelines on Euthanasia.
- 2.2. Does not apply to depopulation.
- 2.3. For guidance regarding:
 - 2.3.1. Use of MS222, see TAMU-G-021.
 - 2.3.2. Zebrafish, see TAMU-G-008

3. RESPONSIBILITY

- 3.1. The **PI** is responsible for:
 - 3.1.1. Ensuring that any euthanasia method that deviates from the AVMA Guidelines on Euthanasia is justified for scientific or medical reasons and is described in the approved animal use protocol.
 - 3.1.2. Ensuring that animals are euthanized by trained personnel using appropriate technique, equipment and agents, as outlined in the approved animal use protocol.
 - 3.1.3. Describing use of the guillotine or scissors in the approved animal use protocol and ensuring that anyone using the equipment is properly trained.
 - 3.1.3.1. Ensuring the equipment used for decapitation is clean, sharp and in good working order.
 - 3.1.3.2. The PI will create an SOP for ensuring guillotine sharpness.
 - 3.1.4. Training personnel to minimize distress and to recognize and confirm death.
 - 3.1.5. Documenting training according to TAMU-G-029.
- 3.2. The **IACUC** is responsible for:
 - 3.2.1. Reviewing and approving methods of euthanasia including scientifically justified methods that deviate from the current AVMA Guidelines on Euthanasia.
- 3.3. The **AWO** staff is responsible for:
 - 3.3.1. Notification of PI when competency certification is required as a part of the administrative review for protocols and amendments proposing physical methods of euthanasia without pre-sedation as described in TAMU-G-029.

4. DEFINITIONS AND/OR ACRONYMS

- 4.1. **Acceptable:** A method considered to reliably meet the requirements of euthanasia. See EUTHANASIA.
- 4.2. **Acceptable With Conditions:** A method considered to reliably meet the requirements of euthanasia when specified conditions are met. See EUTHANASIA.
- 4.3. **AV:** Attending Veterinarian. Individual designated by Texas A&M University to fulfil the regulatory role of AV. May also describe veterinary staff who report directly to, and have delegated authority from, the AV.
- 4.4. **Aversion:** A desire to avoid or retreat from a stimulus
- 4.5. **AVMA:** American Veterinary Medical Association. Nation’s leading advocate for the veterinary profession through a variety of avenues including education programs and the provision of position statements on key issues including humane euthanasia, i.e. AVMA Guidelines for the Euthanasia of Animals.
- 4.6. **AWO:** Animal Welfare Office. Supports the IACUC administratively.

- 4.7. **Euthanasia:** A method of humane destruction that minimizes pain, distress, and anxiety experienced by the animal prior to loss of consciousness, and causes rapid loss of consciousness followed by cardiac or respiratory arrest and death.
- 4.8. **DEA:** United States Drug Enforcement Agency
- 4.9. **Depopulation:** The rapid destruction of a population of animals in response to urgent circumstances with as much consideration given to the welfare of the animals as practicable.
- 4.10. **Distress:** The effect of stimuli that initiate adaptive responses that are not beneficial to the animal—thus, the animal’s response to stimuli interferes with its welfare and comfort.
- 4.11. **FDA:** U.S. Food and Drug Administration
- 4.12. **Guidance:** Guidance documents are developed by the IACUC to provide procedural standards to the research community on the topics identified. Animal care and use program participants are expected to adhere to the standards described unless an exception has been requested and approved by the IACUC.
- 4.13. **IP:** Intraperitoneal
- 4.14. **IV:** Intravenous
- 4.15. **MS222:** Tricaine Methanesulfonate is an FDA-approved drug (“Tricaine-S”) for temporary immobilization (sedation, anesthesia) of finfish, amphibians, and other aquatic, cold-blooded animals.
- 4.16. **Non-inhaled Agents of Euthanasia:** Include chemical agents that are introduced into the body by means other than through direct delivery to the respiratory tract. The primary routes of their administration are parenteral injection, topical application, and immersion.
- 4.17. **Secondary Method:** A euthanasia method employed subsequent to a primary method to ensure death of an unconscious animal before it can recover consciousness.
- 4.18. **Unacceptable:** A method that does not meet the requirements of euthanasia.

5. GUIDELINES OR PROCEDURE

- 5.1. While there is ongoing debate about the ability of fish, amphibians, and reptiles to feel pain or otherwise experience compromised welfare, they do respond to noxious stimuli. Consequently, the IACUC assumes a conservative and humane approach to the care of all vertebrate.
- 5.2. See TAMU-G-010 for additional information on the use, labelling and storage of compounds.

5.3. Fish

5.3.1. Injectable Agents

- 5.3.1.1. Sodium pentobarbital (60 to 100 mg/kg [27.3 to 45.5 mg/lb]) can be administered by IV, intracardiac, or intracoelomic routes for euthanasia.
- 5.3.1.2. Pentobarbital may also be administered via intracardiac injection for anesthetized animals as the second step of a 2-step euthanasia procedure.

5.3.2. Rapid Chilling

- 5.3.2.1. **Note:** Rapid chilling of fish is **not** the same as rapid freezing. Rapid freezing of conscious fish is not considered humane.
- 5.3.2.2. Species-specific thermal tolerance and body size will determine the appropriateness and effectiveness of rapid chilling for euthanasia of fish.
- 5.3.2.3. Fish size is important because the rate of heat loss via thermal conduction from a body is proportional to its surface area.
- 5.3.2.4. Based on these two factors, it has been suggested that rapid chilling in water associated with an ice slurry is a suitable euthanasia method for small tropical and subtropical fish species 3.8 cm in length (tip of the snout to the posterior end of the last vertebra) or smaller, having lower lethal temperatures above 4°C.

- 5.3.2.5. To ensure optimal hypothermal shock (i.e., rapid euthanasia), transfer of fish into ice water must be completed as quickly as possible. This means rapid transition from acclimatization temperature to 2° to 4°C must be achieved. This can be accomplished by using minimal water volume to transfer fish (i.e., using a net to place fish in chilled water).
- 5.3.2.6. Fish should not be in direct contact with the ice in the water; rather a depression should be formed in the ice slurry to expose the entire surface of the fish to the chilled water. Full contact with cold water ensures optimal exposure and rapid chilling of the fish.
- 5.3.2.7. Water temperature must not exceed 2° to 4°C. Well-insulated containers, such as coolers, will assist in maintaining the ice slurry and a probe thermometer can be used to confirm water temperature.
- 5.3.2.8. May be followed by an approved secondary euthanasia method, per protocol.
- 5.3.2.9. Use of a dilute sodium hypochlorite or calcium hypochlorite solution may be an adjunctive method for early life stages of fish, e.g.: embryos and larvae only. Scientific justification must be provided in the animal use protocol for later stages in fish.

5.3.3. Immersion

5.3.3.1. MS222

- 5.3.3.1.1. MS222 is not FDA approved for use as an agent of euthanasia. It is described as an extra-label euthanasia agent in fish in the AVMA Guidelines for the Euthanasia of Animals, 2020 Edition.
- 5.3.3.1.2. Immersion of fish in buffered solutions of MS222 for 30 minutes following loss of rhythmic opercular movement is sufficient for euthanasia of most fish. Due to species differences in response to MS222, a secondary method of euthanasia is recommended in many fish to ensure death.
- 5.3.3.1.3. A concentration of 250 to 500 mg/L, or 5 to 10 times the anesthetic dosage, is effective for most species.
- 5.3.3.1.4. MS222 solutions **must** be buffered. See TAMU-G-021 for guidance on using this agent.

5.3.3.2. Clove oil and its derivatives (isoeugenol and eugenol)

- 5.3.3.2.1. Clove oil and its derivatives are not FDA approved for use as an agent of euthanasia. These are described as extra-label euthanasia agents in fish in the AVMA Guidelines for the Euthanasia of Animals, 2020 Edition.
- 5.3.3.2.2. These agents are not acceptable means of euthanasia for animals intended for consumption.
- 5.3.3.2.3. Whenever possible, use products with standardized, known concentrations of essential oils for accurate dosing.
- 5.3.3.2.4. These oils are not very water soluble; injecting the solution through a syringe and fine-gauge needle under the water in the container used for euthanizing is helpful in ensuring dispersal in the water.
- 5.3.3.2.5. Fish should be left in the anesthetic solution for a minimum of 10 minutes after cessation of opercular movement.
- 5.3.3.2.6. Some clove oil derivatives are potential carcinogens.
- 5.3.3.2.7. For small tropical fishes, 0.20 mL of clove oil has been recommended for studies that require euthanasia.

5.3.4. Secondary Methods

5.3.5. Decapitation, pithing, exsanguination, or freezing may be used as the second step of a 2-step procedure when fish have been rendered unconscious prior to their application by an acceptable or acceptable-with-conditions, first-step method.

5.4. Amphibians and Reptiles

5.4.1. Amphibians and reptiles represent taxa with a diverse range of anatomical and physiological characteristics such that it is often difficult to ascertain that an amphibian or reptile is, in fact, dead.

5.4.2. Euthanasia techniques that result in “rapid loss of consciousness” and “minimize pain and distress” should be strived for, even where it is difficult to determine that these criteria have been met.

5.4.3. Immersion

5.4.3.1. Tricaine methanesulfonate (MS222) is an acceptable method of euthanasia for some amphibians and reptiles, though is not FDA approved for use as an agent of euthanasia as noted in Fish above. See TAMU-G-021 for guidelines on its use.

5.4.3.2. Alternatives should be considered with terrestrial species as drowning is an unacceptable method of euthanasia.

5.4.3.3. Immersion for a minimum of 30 minutes is suggested, however, immersion for as long as 1 hour may be required. Immersion should be followed by a secondary method of euthanasia, such as double pithing, or as described in the approved protocol.

5.4.3.4. For *Xenopus laevis*: If a concentration of MS222 < 5g/L or shorter time frame than 1 hour is allowed, a secondary euthanasia method should be used.

5.4.3.5. In Leopard frogs, one study found that immersion in a 310–318 mg/L clove oil solution induced widely variable anesthesia within 15 minutes.

5.4.4. Inhaled Agents

5.4.4.1. Reptiles and amphibians can have a great capacity for holding their breath and for anaerobic metabolism. Due to these respiratory adaptations, reptiles and amphibians may breathe too slowly for the use of CO₂ or other inhaled agents.

5.4.5. Injectable Agents

5.4.5.1. Barbiturates are best administered intravenously to minimize the discomfort upon injection. However, where intravenous administration is not possible or its benefits are outweighed by distress imposed by additional restraint, pain from alternate methods, risk to personnel, or other similar reasons, intracoelomic administration is an acceptable route for administration of barbiturates.

5.4.5.2. Amphibian species commonly used in research include the African and Western clawed frog (*Xenopus laevis*, *Xenopus tropicalis*), leopard and bull frogs (*Rana* spp.), and axolotls (*Ambystoma mexicanum*). These species are best euthanized via a physical method while fully anesthetized. While injection of sodium pentobarbital IV, intracelomically, or in the lymph spaces is an acceptable method of euthanasia of these species, high doses are often required and these agents may have an inconsistent time to loss of consciousness.

5.4.5.3. Buffered MS222 may be injected directly into the lymph sacs (amphibians) or the coelomic cavity (small amphibians and reptiles), as appropriate per species (as a primary or secondary method).

5.4.5.3.1. Intracoelomic injection of MS222 is **not** an acceptable method of euthanasia for *X. laevis*.

5.4.5.4. A 2-stage euthanasia method for reptiles using MS222 has been described.

5.4.5.4.1. The first stage entails intracoelomic injection of 250 to 500 mg/kg (113.6 to 227.3 mg/lb) of a pH-neutralized solution (0.7% to 1.0% MS222), which results in rapid loss of consciousness (< 30 seconds to 4 minutes).

5.4.5.4.2. Once unconsciousness occurs, a second intracoelomic injection of unbuffered 50% MS222 is administered.

5.4.6. Topical

5.4.6.1. The application of benzocaine hydrochloride gel to the ventral abdomen of amphibians (20% concentration; 2.0-cm X 1.0-mm application) is an effective means of anesthesia and euthanasia for some species.

5.4.6.2. Currently there are no topical euthanasia agents that are FDA approved for any species.

5.4.7. Decapitation

5.4.7.1. Those responsible for the use of this method must ensure that personnel who perform decapitation have been properly trained to do so and are monitored for competence.

5.4.7.2. Training records documenting competency of the performance of physical methods of euthanasia must be maintained in the laboratory as outlined in TAMU-G-029.

5.4.7.3. Training records, equipment, SOPs for assessing sharpness, as well as maintenance logs will be inspected as part of the IACUC semi-annual inspections.

5.4.7.4. It has been assumed that stopping blood supply to the brain by decapitation causes rapid loss of consciousness. However, because the CNS of reptiles and amphibians is tolerant to hypoxic and hypotensive conditions, decapitation must be followed by pithing or another method of destroying brain tissue.

5.4.7.5. Decapitation should only be performed as part of a 3-step euthanasia protocol (injectable anesthetic, decapitation, pithing).

5.4.8. Pithing

5.4.8.1. Can be used as a second-step euthanasia method in unconscious animals when performed by properly trained individuals.

5.4.8.2. The pithing site in frogs is the foramen magnum, and it is identified by a slight midline skin depression posterior to the skull, midline between the eyes, with the neck flexed.

5.4.9. Recommendations (Amphibians/Reptiles)

5.4.9.1. Non-inhaled methods of euthanasia should be considered for these species and a secondary method employed to euthanize the unconscious animal.

5.5. Verification of Death

5.5.1. Fish

5.5.1.1. Because the thousands of species of fish vary greatly in anatomical and physiological characteristics, reliable indicators of death may not be available for some.

5.5.1.2. Standard indicators of death of fish include: loss of movement, loss of reactivity to any stimulus, and initial flaccidity (prior to rigor mortis).

5.5.1.3. More useful indicators for many fish include: respiratory arrest (cessation of rhythmic opercular activity) for a minimum of 30 minutes and loss of eyeroll (vestibulo-ocular reflex, the movement of the eye when the fish is rocked from side to side). The latter is no longer present in fish that have been deeply anesthetized or euthanized.

- 5.5.1.4. Note: The heart can continue to contract even after brain death or removal from the bodies of fish, so the presence of a heartbeat is not a reliable indicator of life, but sustained absence of heartbeat is a strong indicator of death.
- 5.5.1.5. For more sessile, less active fish, or those with specific anatomical or physiological adaptations that prevent use of these indicators, it may be more difficult to assess loss of consciousness and death, and consultation with species experts is recommended.
- 5.5.1.6. Secondary methods of euthanasia are recommended, when appropriate, after the fish is anesthetized, to ensure euthanasia.

5.5.2. Amphibians and Reptiles

- 5.5.2.1. Methods used to verify death in mammalian species, such as auscultation, ECG, Doppler ultrasound, or pulse oximetry, can be used for amphibians and reptiles, but it is important to remember that amphibian and reptilian hearts can beat even after brain death. Death should always be confirmed by physical intervention.

6. EXCEPTIONS

- 6.1. The PI may request an exception to the above standards by describing the departure in the AUP
- 6.2. For programmatic exceptions, the facility director or manager may submit a request for the exception using TAMU-F-013.

7. REFERENCES, MATERIALS, AND/OR ADDITIONAL INFORMATION

- 7.1. References
 - 7.1.1. American Veterinary Medical Association. [AVMA Guidelines for the Euthanasia of Animals: 2020 Edition.](#)
 - 7.1.2. Fernandes, IM et al. The efficacy of clove oil as an anaesthetic and in euthanasia procedure for small-sized tropical fishes. [Braz J Biol.](#) 2017 Jul-Sept;77(3):444-450.
 - 7.1.3. Institute for Laboratory Animal Research. Guide for the Care and Use of Laboratory Animals, 8th Edition. National Academies Press, 2011.
 - 7.1.4. CITI:
 - 7.1.4.1. Web page: <https://about.citiprogram.org/en/homepage/>
 - 7.1.4.2. Instructions: <https://rcb.tamu.edu/animals/training>
 - 7.1.4.3. CITI Working with Amphibians in Research Settings
 - 7.1.4.4. CITI Working with Reptiles in Research Settings
- 7.2. Resources
 - 7.2.1. American Fisheries Society [Guidelines for the Use of Fishes in Research](#)
 - 7.2.2. IACUC/AWO Referenced Documents [requires TAMU NetID authentication]: <https://rcb.tamu.edu/animals/guidance>
 - 7.2.2.1. TAMU-F-013 Request for Programmatic Exception from Animal Welfare Standards
 - 7.2.2.2. TAMU-G-008 Guidelines on Working with Zebrafish
 - 7.2.2.3. TAMU-G-010 Guidelines for the Use of Pharmaceutical and Non-Pharmaceutical Grade Drugs and Compounds
 - 7.2.2.4. TAMU-G-021 Guidelines for the use of MS222
 - 7.2.2.5. TAMU-G-029 Guidelines for Animal Protocol Participation and Handling
 - 7.2.2.6. TAMU-G-035 Guidelines on Performing Surgery in Non-mammalians

8. HISTORY

Effective Date	Version #	Description
09/01/2021	000	College Station/Galveston: Updated content and new format. Replaces previous un-



		numbered document entitled, "Ice Water Immersion for Euthanasia of Small Tropical and Subtropical Fish"
03/24/2022	001	College Station/Dallas/Galveston: Merging of Dallas animal care and use program with College Station/Galveston
06/01/2022	002	College Station/Galveston: Renewal; updated responsibilities and references. Included info related to decapitation (competency training and equipment maintenance)
10/20/2022	003	College Station/Dallas/Galveston/Kingsville: Merging of Kingsville animal care and use program with College Station/Dallas/Galveston.