

**STANDARD OPERATING PROCEDURES**  
**DIVISION OF COMPARATIVE MEDICINE**  
**UNIVERSITY OF SOUTH FLORIDA**

SOP#: 806.2

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**TITLE:** Fish, Amphibian, and Reptile Euthanasia  
**SCOPE:** Animal Care Personnel  
**RESPONSIBILITY:** Facility Manager, Animal Care, and Research Personnel  
**PURPOSE:** To Outline the Proper Procedures for Performing Euthanasia in Aquatic Species

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**I. PURPOSE**

1. This procedure outlines the proper methods for euthanasia of fish, amphibians, and reptiles.

**II. RESPONSIBILITY**

1. The Veterinarians oversee all aspects of animal health, and are assisted by all program staff.
2. Facility Managers ensure implementation of all procedures.
3. Research staff are required to follow these guidelines.

**III. PROCEDURES**

1. **Tricaine methanesulfonate (MS-222, TMS)** is an acceptable method of euthanasia according to the AVMA Guidelines on Euthanasia (2020 edition)
  - a. Immersion
    1. MS-222 is acidic in solution and must be buffered by adding an equal weight of sodium bicarbonate or titrating to pH=7.0-7.5.
    2. Immerse animal in buffered solution of MS-222 at a concentration of:

Species	Concentration	Duration
Fish	at least 250mg/L	At least 30 minutes
Amphibians	2-5g/L (most species)	Up to 1hr

3. **Death must be confirmed** by observing cessation of opercular movements for at least 30 minutes and/or by performing a physical method (i.e., pithing or decapitation) of euthanasia.
- b. Injection
    1. Buffered MS-222 may be injected directly into the lymph sacs (amphibians) or coelomic cavity (reptiles).
    2. As reptile and amphibian hearts can continue to beat even after brain death, death must be confirmed by utilizing a secondary physical method of euthanasia (pithing, decapitation).

- c. MS-222 is light sensitive and stock solution should be refrigerated. Stock preparations should be discarded **at least monthly** or if solution becomes brown in color.
  
- d. **MS-222 safe practices**
  1. Wear appropriate PPE, to include (at a minimum) nitrile gloves and appropriate eye protection that provides splash protection when handling MS-222 powder.
  2. If possible, work inside a fume hood to prepare a concentrated stock. Mix MS-222 powder in a volume of water appropriate to obtain the desired concentration based on the manufacturer's recommendations.
  3. Wear gloves and use a utensil until all powder is dissolved.
  4. Dispose of MS-222 waste via flushing with excess amounts and in accordance with recommendations provided by Environmental Health and Safety (EHS).
  
2. **Benzocaine-hydrochloride** is an acceptable method of euthanasia as appropriate by species according to the AVMA Guidelines of Euthanasia (2020 edition).
  - a. Benzocaine-HCL is acidic in solution and must be buffered by adding an equal weight of sodium bicarbonate or titrating to pH 7.0-7.5.
    1. Solution for immersion should be prepared in concentrations of at least 250 mg/L
      - a. Place animal into a bath containing buffered Benzocaine-HCl and immerse until death.
    2. Alternatively, topical Benzocaine gel at a concentration of 7.5 % or 20% can be applied to the ventral abdomen of amphibians and does not require buffering.
    3. Time to effect is proportional to concentration and aquatic species but this method of euthanasia may require at least 3 hours before death results.
  - b. Benzocaine-HCL is light sensitive and should be protected from freezing and or excessive heat.
  - c. **Death must confirmed** observing cessation of opercular movements for at least 10 minutes and/or by performing a physical method (i.e. pithing or decapitation) of euthanasia.
  
3. **Rapid Cooling** is an acceptable method of euthanasia **for adult zebrafish only** according to the AVMA Guidelines of Euthanasia (2020 edition).
  - a. Prepare ice water bath of 1:1 ice: system water. Add ice as needed to ensure temperature is between 0–4°C. Ensure fish do not come into direct contact with ice.
  - b. Transfer fish to ice water bath with a net, no more than 25 fish per liter.
  - c. To ensure death, fish must be maintained in the chilled water for at least 10 minutes after opercular movement has ceased. If it is difficult to observe opercular movement, fish should be left in ice water for at least 20 minutes after cessation of all movements to ensure death by hypoxia.
  
4. **Other injectables**

- a. Sodium pentobarbital (60-100 mg/kg of body weight) can be administered IV, intracoelomically, into subcutaneous lymph spaces, or into the lymph sacs.
  1. Dosages vary widely by species and the veterinary staff and appropriate literature should be consulted for guidance.
  2. Death must be confirmed by utilizing a secondary physical method of euthanasia (pithing, decapitation).
- b. Dissociative agents (ketamine, Telazol), other IV administered anesthetics (propofol) may be used to induce rapid general anesthesia and subsequent euthanasia, but death must be confirmed by utilizing a secondary physical method of euthanasia (pithing, decapitation).

## 5. References

- a. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition
- b. Bowker, J.D., J.T. Trushenski, M.P. Gaikowski, and D.L. Straus, Editors. 2012. Guide to Using Drugs, Biologics, and Other Chemicals in Aquaculture. American Fisheries Society Fish Culture Section. euthanasia: Considerations, methods, and types of drugs. ILAR Journal, 50(4), 343-360.
- c. JW Carpenter, CJ Marion, eds. (2013): Exotic Animal Formulary, 4<sup>th</sup> Edition. Elsevier Saunders, St. Louis.
- d. Neiffer, D.L., Stamper, M.A. 2009. Fish sedation, anesthesia, analgesia, and
- e. Richard E. Fish, Peggy J. Danneman, Marilyn Brown, and Alicia Z. Karas, eds. (2008): *Anesthesia and Analgesia in Laboratory Animals*, second edition

**Approved:**

**Date:**