

STANDARD OPERATING PROCEDURES
DIVISION OF COMPARATIVE MEDICINE
UNIVERSITY OF SOUTH FLORIDA

SOP#: 806

Date Issued: 12/18

Date Revised: New

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TITLE: Fish and Amphibian Euthanasia using Tricaine and Benzocaine-HCl
SCOPE: Animal Care Personnel
RESPONSIBILITY: Facility Manager, Animal Care, and Research Personnel
PURPOSE: To Outline the Proper Procedures for Performing Euthanasia in Aquatic Species using Tricaine and Benzocaine-HCL

I. PURPOSE

1. This procedure outlines the proper methods for euthanasia of fish and amphibians using Tricaine and Benzocaine HCl

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 (2013 edition)
 - a. 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.
 1. Immerse animal in buffered solution of MS-222 at a concentration of:

Species	Concentration	Duration
Fish	at least 250mg/L	At least 10 minutes
Amphibians	2-5g/L (most species)	Up to 1hr

2. **Death must be confirmed** by observing cessation of opercular movements for at least 10 minutes and/or by performing a physical method (i.e., pithing or decapitation) of euthanasia.
 - b. 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.
2. **Personnel Safety**
 - a. MS-222 safe practices

1. Wear appropriate PPE 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 manufacturers 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).
3. **Benzocaine-hydrochloride** is an acceptable method of euthanasia as appropriate by species according to the AVMA Guidelines of Euthanasia (2013 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.

4. References

- a. Amphibian Medicine and Captive Husbandry, KM Wright & BR Whitaker, 2001; Krieger Publishing Company, Malabar, FL
- b. AVMA Guidelines for the Euthanasia of Animals: 2013 Edition
- c. 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.
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- e. Neiffer, D.L., Stamper, M.A. 2009. Fish sedation, anesthesia, analgesia, and
- f. Richard E. Fish, Peggy J. Danneman, Marilyn Brown, and Alicia Z. Karas, eds. (2008):
Anesthesia and Analgesia in Laboratory Animals, second edition

Approved:

Date: