



OFFICE OF
**RESEARCH
COMPLIANCE**
CENTRAL MICHIGAN UNIVERSITY

Title	Euthanasia of Amphibians and Fish using MS-222 SOP
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Authorized by	IACUC

I. PURPOSE

To define a set of approved procedures for performing euthanasia on amphibians and fish using Tricaine Methanesulfate (MS-222). MS-222 is an anesthetic agent that may be used to anesthetize and euthanize amphibians and fish. This standard operating procedure provides the euthanasia method and procedural details in accordance with the IACUC Euthanasia Policy.

The PHS Policy requires that euthanasia be conducted according to the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals (2020 Edition). The AVMA Guidelines set criteria for euthanasia and specify appropriate euthanasia methods and agents based upon the best literature and empirical evidence that minimizes pain and distress to animals during euthanasia.

II. POLICY

Per the IACUC Euthanasia Policy, “Methods not covered in approved IACUC standard operating procedures need to be approved by the IACUC before utilization. The Principal Investigator should confer with the Attending Veterinarian prior to submitting the protocol to the IACUC.”

III. DEFINITIONS

Euthanasia – In the context of this SOP, euthanasia is the act of inducing humane death in an animal with rapid unconsciousness and death with a minimum of pain, discomfort, or distress.

IV. PROCEDURES

A. General:

1. All individuals responsible for euthanasia of research animals must be qualified and trained to perform euthanasia humanely, per the IACUC policy on Training for Research and Animal Care Personnel (IACUC-003).
 - a. The Principal Investigator is responsible for ensuring that their personnel have been sufficiently trained, and for maintaining appropriate documentation.
 - b. Documentation of personnel training may be reviewed by the IACUC.
2. These procedures must be posted near the routine area of or the MS-222 storage location(s).
3. Euthanasia procedures should be documented. See Appendix A for an example.
4. Death must be confirmed by a second physical form of euthanasia for narcotic or inhalant euthanasia processes. Examples:
 - a. Decapitation
 - b. Pithing

- c. Doppler
 - d. Freezing
 - e. $\geq 75\%$ EtOH (ethanol/ethyl alcohol)
5. Animals must not be left alone at any point during the euthanasia processes.

B. Hazards and Safety Precautions:

- 1. MS-222 may be irritating to humans by inhalation, ingestion, or absorption through the skin.
- 2. When preparing a stock solution using the powder form of MS-222, all lab work will be done under a chemical fume hood. Researchers should prepare stock solutions prior to leaving the lab areas for field work to ensure appropriate protections are in place.
- 3. PPE
 - a. Lab coat
 - b. Splash goggles or a face shield with splash goggles
 - c. Nitrile gloves

C. Storage and Expiration:

- 1. Follow the manufacturer's printed expiration date. If unspecified, it should be marked to expire 3 years after receipt.
- 2. Store in a cool, dry place away from light.
- 3. Frozen ($-20\text{ }^{\circ}\text{C}$) stock solutions have a shelf-life of six months and should be stored in opaque, sealed containers.
- 4. Refrigerated ($4\text{ }^{\circ}\text{C}$) stock solutions must be replaced by 30 days.
- 5. Discard or replace powder or stock solutions by the expiration date.
- 6. Discard stock solutions if the liquid begins to show brown coloration – regardless of the expiration date.
- 7. Containers must be labeled to indicate contents, hazards, concentration, and the expiration date.

D. Buffering

- 1. Unbuffered MS-222 solution is acidic and irritating to amphibians, fish, especially via the thin gill epithelium, and to other vertebrates.
- 2. When using MS-222 solutions in the lab or field settings, solution must be buffered to a pH between 7.0 and 7.5 using sodium bicarbonate.

E. Use of MS-222**1. Amphibians**

- a. Use a solution containing 500 mg/L powdered MS-222 in one liter of tap water.
Note: If using a stock solution of MS-222, dilute the solution to obtain an equivalent concentration of MS-222.
- b. Buffer to a pH between 7.0 and 7.5 as noted in section IV.D.
- c. Anesthetize by immersing the amphibian in solution for ten minutes.
- d. Test the level of anesthesia by evaluating leg retraction reflexes and response to toe pinch.
- e. A secondary step must be conducted for euthanasia.
Examples: decapitation, pithing

2. Fish

- a. Concentrations are highly species dependent.
- b. Typically, use MS-222 at a concentration of 350 mg/L for euthanasia of fish.
- c. Buffer to a pH between 7.0 and 7.5 as noted in section IV.D.
- d. Keep fish immersed for at least 10 minutes after cessation of opercular movements. Depending upon species, additional time may be warranted.

- e. A secondary step must be conducted to ensure confirmation of death.
Examples: decapitation, pithing, Doppler, freezing

F. Disposal

1. Death must be confirmed prior to bagging carcasses for disposal.
2. Animal carcasses must be disposed of properly via biohazardous waste disposal.
3. Disposal of MS-222:
 - a. MS-222 is disposed of as hazardous waste using an appropriate waste disposal company.
 - b. MS-222 used in field settings must be returned to campus for appropriate disposal.

V. REFERENCES

American Veterinary Medical Association, Panel on Euthanasia. (2020). *AVMA guidelines for the euthanasia of animals: 2020 edition*. American Veterinary Medical Association.
<https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf>

Appendix A: Example - Euthanasia Log

Amphibian & Fish Euthanasia Record

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