

<b>Title: Euthanasia of Amphibians and Fish using MS-222, IACUC Standard Operating Procedure</b>	
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<b>Authorized by:</b> Vice President for Research and Innovation, and the Institutional Animal Care and Use Committee (IACUC)	
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## 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 Policy on Euthanasia.

The PHS Policy requires that euthanasia be conducted according to the American Veterinarian Medical Association (AVMA) Guidelines for 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 IACUC Policy on Euthanasia, “Methods not covered in approved IACUC standard operating procedures need to be approved by the IACUC. The Principal Investigator should confer with the attending or clinical veterinarian prior to submitting the protocol to the IACUC. The IACUC committee must grant an exemption to any deviation from approved policies and procedures.”

## III. DEFINITIONS

**Euthanasia** – In the context of this policy, 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 training policy (P-003-02).
  - a. The Principal Investigator is responsible for ensuring appropriate documentation.
  - b. Documentation of personnel training may be reviewed by the IACUC.
2. These procedures must be posted near the routine area of use or the MS-222 storage location(s).
3. There should be a log used for tracking euthanasia procedures or MS-222 use, see Appendix A for example.
4. Confirmation of death must be ensured by a second form of euthanasia for narcotic or inhalant euthanasia processes. Examples:
  - a. Decapitation
  - b. Pithing
  - c. Doppler
  - d. Freezing

- e.  $\geq 75\%$  EtOH
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
  - c. Nitrile gloves

**C. Storage and Expiration:**

1. Powdered MS-222 has a shelf-life of one year (or per manufacturer recommendations).
2. Stock solutions have a shelf-life of six months and should be stored in opaque, sealed containers.
3. Containers must be labeled to indicate contents, hazards, concentration, and the expiration date.
4. Discard or replace powder or stock solutions by the expiration date.
5. Discard stock solutions if the liquid begins to show brown coloration – regardless of 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 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 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>

