

**Title:** The Use of Tricaine Methanesulfonate (MS-222) in Fishes and Other Aquatic Animals **Policy Number:** 031 **Policy Intent:** The intent of this policy is to describe the handling, preparation and use of MS-22

**Policy Intent:** The intent of this policy is to describe the handling, preparation and use of MS-222 at Texas Tech University.

### Table of Contents

- 1. Purpose
- 2. Usage Policy
- 3. References

#### 1. Purpose

A. The purpose of this policy is to describe the recommended methods of handling, preparation and use of MS-222. Tricaine methanesulfonate, also called MS-222 or TMS among other names, is used as an anesthetic and euthanasia agent in fish, amphibians, and other cold-blooded animals. This document provides guidance regarding safety for personnel preparing MS-222 solutions, the proper usage of the compound, dosages recommended for different applications and proper disposal.

# 2. Usage Policy

#### A. Formulation

1) The IACUC strongly recommends a pharmaceutical grade MS-222 product. If other MS-222 products are proposed to be utilized, the IACUC will request a justification why a non-pharmacological compound has to be used (see also IACUC Policy 14).

### **B. Safety Precautions**

- It is the responsibility of the PI on the corresponding animal use protocol to assure the safe use of MS-222 by laboratory staff. The PI is responsible for the appropriate training of research personnel for the safe use of this anesthetic/euthanasia agent in the species approved on the protocol. Personnel using MS-222 should be familiar with the SDS, which should be readily available in the lab. Questions regarding safety practices should be directed to <u>TTU Environmental Health & Safety</u>.
- 2) MS-222 is considered an irritant to the eyes, respiratory system and skin; safety precautions per the <u>University Laboratory Safety Manual</u> must to be employed. Accidental ingestion or exposure to the blood stream may be damaging to the health of the individual by inducing anoxia. Symptoms may not be evident until several hours after exposure.
- 3) To prevent exposure, the powder should be weighed in a fume hood. To avoid skin and eye contact, goggles, gloves and a lab coat/protective clothing should be worn while measuring the powder. Avoid aerosolizing the powder during handling.
- 4) In typical use, the mode of action is by absorption through the skin of aquatic animals. It can also permeate human skin although at a much lower rate, particularly when dissolved. Personnel should therefore minimize contact with medicated water whenever possible and should be aware and attentive to nausea or headaches after any prolonged exposure of even low concentrated MS-222 solution.



- 5) When working with MS-222 solutions in an anesthetizing bath, personnel should consider the use of additional PPE to address any splash hazard such as liquid repellent arm covers, splash goggles or liquid repellent apron.
- 6) In situations where large quantities or prolonged exposure occur, users should consult with TTU Environmental Health & Safety.

# C. Preparation

- 1) MS-222 comes in a powdered form and should be stored at room temperature, in a cool dry place protected from light.
- 2) Use of freshly prepared solutions is required, especially if used for surgical procedures to minimize contamination and infection. It has been reported that after 10 days, a 10 percent solution showed a 5% decrease in potency. When used for surgical procedures, preparations must be no more than 48 hours old to ensure potency. All preparations must be discarded within 10 days.
- 3) A stock solution can be prepared for use in water bath. Commonly used MS-222 stock solutions are at 10 g/L using aged tap water. Sodium Bicarbonate should be added (10-20g/L) resulting in a pH between 7.0 and 7.5.
- 4) Containers must be labeled with the agent, concentration, and date of preparation.
- 5) Store preparation at -20C in a dark brown bottle or use tinfoil around the bottle. Discard frozen preparations after one month or earlier if an oily film or discoloration of the stock solution develops.
- 6) Dry MS-222 and dry Sodium Bicarbonate should be stored in separate containers since premixed dry ingredients can become hydroscopic and react reducing the effectiveness of the solution.

### D. Use as an Anesthetic

- 1) Solutions for bath immersion are acidic and irritating and must be buffered with sodium bicarbonate to a normal pH (7.4) before use.
- 2) The action of MS-222 as an anesthetic varies widely between species and is affected by water temperature, hardness, and size of the individual animal. Preliminary tests are recommended to determine concentration and exposure time for each application to assure sufficient anesthetic depth and safe recovery.
- 3) Allow animal to reach appropriate level of anesthesia for planned procedure.
- 4) In amphibians, anesthesia induction can be in a water bath. When inducing a terrestrial amphibian in an immersion anesthetic bath, keep the animal's head and nares above the water line to prevent accidental drowning. In some cases, anesthesia with MS-222 can be maintained by dripping a dilute solution of this drug over the skin or by covering animals with a paper towel moistened with the anesthetic.
- 5) MS-222 is the preferred anesthetic agent for amphibians and can be administered via immersion in a buffered solution or by intracoelomic injection.
  - > 0.1-0.5% (1-5 g/L) for adult Xenopus
  - > 0.1-0.2 g/L for tadpoles
- 6) Tricaine is the only FDA anesthetic approved in the U.S. for fish intended for food and is the most commonly used agent for the anesthesia of fish.
  - 25-100 mg/L (Zebrafish 100 mg/L)
- 7) After procedures are completed, place animals in well oxygenated/aerated, unmedicated water or in a container lined with wet towels (terrestrial amphibians).



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8) Closely monitor fish/amphibians recovering from anesthesia until they are swimming/moving normally and completely regained their righting response.

# E. Euthanasia

- 1) Fish
  - Based on life stage, MS-222 (observe appropriate pH see preparation section) may be used to euthanize fish,. It is considered an acceptable method per AVMA Guidelines on Euthanasia (2020 edition) in zebrafish >15 dpf.
    - 1. Juvenile and Adult fish need to be immersed in buffered MS-222 (250-500 mg/L) and kept in the solution for at least 30 min following cessation of opercular movement. Potency is increased in warm water and decreased in cold water.
  - For zebrafish larvae up to 8-15 dpf, euthanasia requires a secondary method in order to ensure death.
    - 1. Two step euthanasia method- Pharmaceutical grade MS-222 immersion followed by adjunctive method
      - a. Decapitation
      - b. Freezing
      - c. Other physical or chemical methods for destroying brain function
  - 2) Amphibians
    - Prolonged immersion (> 1 hour) in a buffered solution of MS-222 (10g/L, pH 7 to 7.5) is effective for most amphibian euthanasia. However, the technique may not be completely effective in some species such as the Smokey Jungle Frogs (*Leptodactylus pentadactylus*), and therefore a secondary method is recommended, especially in larger animals or in cooler temperatures
    - Xenopus-Immersion of frogs in 5 g/L of MS-222 results in deep anesthesia with 4 minutes, but at least 1 hour of immersion at this concentration is required to reliably euthanize frogs.
    - Intracoelomic injection of MS-222 is not considered to be an acceptable method of euthanasia for amphibians.
    - Death needs to be assured before discarding the animal, best by following with a secondary method such as pithing or decapitation
  - 3) Reptiles

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- Two-stage euthanasia method
  - Intracoelomic injection of 250-500 mg/kg of a pH-neutralized solution (0.7%-1% MS-222) which results in rapid loss of consciousness. Once unconsciousness occurs, a second intracoelomic injection of unbuffered 50% MS-222 is administered.

# F. Disposal

- 1) Disposal of MS-222 powder and solutions must be in accord with local authority regulations and is administered through TTU Environmental Health & Safety department (EH&S).
- 2) Consult EHS for use of MS-222 in the field.



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# 3. References

- American Veterinary Medical Association (AVMA). (2020). AVMA Guidelines on Euthanasia. <u>https://www.avma.org/KB/Policies/Documents/euthanasia.pdf</u>
- 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.
- Harper, C., C. Lawrence. 2011. The Laboratory Zebrafish.
- Richard E. Fish, Peggy J. Danneman, Marilyn Brown, and Alicia Z. Karas, eds. (2008): Anesthesia and Analgesia in Laboratory Animals, second edition.
- Sherril L. Green. 2010. The Laboratory Xenopus.