



**THE UNIVERSITY OF TOLEDO**  
**INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE**

SUBJECT: Institutional Animal Care and Use Committee (IACUC) Guidelines for the Use of Tricaine Methanesulfonate (MS-222) in Fish

DATE: 7/16/2025

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Tricaine methanesulfonate (MS-222) is used as an anesthetic and euthanasia agent in fish. This document provides guidance regarding the safety of personnel preparing MS-222 solutions, the proper usage of the compound, dosages recommended for different applications, and proper disposal.

**Responsibilities**

- It is the responsibility of the Principal Investigator to include the use of MS-222 in the IACUC protocol or to justify the use of another method of euthanasia.
- It is the responsibility of the Principal Investigator and staff to follow guidelines outlined in this procedure.
- The PI must ensure that all personnel using MS-222 are properly trained and proficient in its use.

**Procedures**

- A. Formulation of MS-222
  - a. The IACUC strongly recommends using a pharmaceutical-grade MS-222 product (Tricaine-S, Syncaïne®); however, if other MS-222 products are to be utilized, justification for the use of a non-pharmaceutical grade compound must be provided in the IACUC protocol.
- B. Safety Precautions
  - a. MS-222 is considered an irritant to the eyes, respiratory system, and skin. Safety precautions should be employed when used.
  - b. To prevent exposure, the powder should be weighed in a fume hood or while wearing an N95 respirator. To avoid skin and eye contact, goggles, gloves and lab coat/protective clothing should be worn. Employees who are required to wear respirators must be cleared through occupational health, to ensure proper mask fitting and testing.
  - c. When handling liquid containing MS222, PPE should include gloves, lab coat and eye goggles to prevent contact with eyes or skin.
- C. Preparation
  - a. MS-222 comes in a powdered form and should be stored at room temperature, in a cool dry place protected from light.
  - b. Ideally MS-222 stock solutions are utilized the same day as preparation per vendor recommendation. When necessary, stock solutions of MS-222 may be kept up to 30 days. They must be refrigerated and stored in tinted (amber) or opaque bottles.
- D. Use as an Anesthetic
  - a. Solutions for bath immersion are acidic and irritating, and therefore, must be buffered with sodium bicarbonate to a normal pH (7.0-7.4) before use.

- b. The action of MS-222 as an anesthetic varies widely between species and is affected by water temperature, hardness, and size of the individual fish. Pilot tests should be conducted to determine concentration and exposure time for each application to assure sufficient anesthetic depth and safe recovery. Dose depends on various factors including species, temperature, exposure time, and recovery time. The regimen should be tailored to experimental conditions through pilot experiments. See the product insert, such as Syncline (R), for specific dose recommendations.
  - c. Dose: The dose, route and frequency should be specified in the IACUC protocol.
  - d. After procedures are completed, place anesthetized fish in well-oxygenated/aerated, un-medicated water.
  - e. Closely monitor fish recovering from anesthesia until they are swimming normally and have completely regained their righting response.
- E. Use for euthanasia
  - a. MS-222 can be used to euthanize fish. It is considered an acceptable method per the AVMA Guidelines on Euthanasia.
  - b. Immerse fish in buffered MS 222 for at least 30 minutes; it is recommended that this is followed by an adjunctive method (decapitation, pithing, exsanguination, freezing).
  - c. A concentration of 250-500 mg/L or 5 to 10 times the anesthetic dose is effective for most species.
- F. Disposal
  - a. Disposal of MS-222 powder and solutions must be done in accordance with local authority regulations, and in consultation with EH&RS.

## Reference

1. *The Guide for the Care and Use of Laboratory Animals*, 8<sup>th</sup> Edition. National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Washington (DC): National Academies Press (US).
2. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition
3. Syndel USA Tricaine-S (tricaine methanesulfonate) product insert. <https://syndel.com/wp-content/uploads/2022/01/Tricaine-S-Insert.pdf>