The University of Pennsylvania Institutional Animal Care and Use Committee (IACUC) has developed the following guideline to help the research community with appropriate preparation and storage of tricaine methanesulfonate (MS-222). It is the responsibility of the Principal Investigator (PI) to institute adequate inventory and laboratory management procedures to ensure that MS-222 is properly prepared, identified, and stored. Deviations from or modifications to the policy must be requested of, and approved by, the IACUC.

This policy offers direction on the following topics:
- Tricaine Methanesulfonate (MS-222) use
- Safe practices for use of MS-222
- MS-222 preparation for use as anesthetic and euthanasia agent
- Storage recommendations
- Dosages for anesthesia and euthanasia of fish and frogs
- Proper disposal of MS-222
- Pharmaceutical grade sources of MS-222

USE
MS-222 is a popular anesthetic agent used in aquatic species, and is intended for the temporary immobilization of fish, amphibians, and other aquatic cold-blooded animals. At much higher doses, MS-222 is also commonly used for euthanasia of aquatic species. It has long been recognized as a valuable tool for the proper handling of these animals during manual spawning (fish stripping), weighing, measuring, marking, surgical operations, transport, photography, and research. Due to the acidity of pharmaceutical grade MS-222, solution must be buffered for all intended uses in live vertebrate aquatic species.

SAFE PRACTICES FOR WORKING WITH MS-222
- Wear protective clothing, gloves and safety glasses when handling the MS-222 powder.
- Work inside a fume hood to prepare a concentrated stock solution by mixing an appropriate amount of MS-222 powder in a small volume of water.
- Dilute the stock solution further as required.
- Wear gloves and use a utensil to stir until all powder is dissolved.
- Wear gloves to handle animals exposed to MS-222.

PREPARATION
“A 10 g/L of stock solution can be made and sodium bicarbonate added to saturation resulting in a pH of 7.0 to 7.5 for the solution. MS-222 is an acceptable method of euthanasia for finfish and for some amphibians and reptiles.” (AVMA)

STORAGE RECOMMENDATIONS
MS-222 powder: Can be stored at room temperature. Discard when expiration date on bottle has been reached.

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Working solution: Store in a bottle protected from light (dark glass, wrapped) and refrigerate at 4°C. Discard remaining solution 4 weeks from the date of preparation, or when degradation of solution has been seen (i.e., brown color occurs).
Concentrated Solutions: Store at -20°C; use the expiration date of the powder from the parent bottle.

ANESTHESIA

Fish
Surgical anesthesia: 100 mg/liter MS-222 buffered to pH 7.0-7.5
Tranquilization and transport: 20-30 mg/liter MS-222 buffered to pH 7.0-7.5
Effective buffering may be achieved by adding food grade baking soda (sodium bicarbonate) in a ratio of 1:1 to MS-222 and water.

At anesthetic doses, (100 mg/liter MS-222), the fish is immersed in the buffered solution (pH 7.0-7.5) and a surgical plane of anesthesia is reached in approximately 2 minutes. The animal can then be removed and anesthesia maintained by dripping solution onto the gills or returning the animal to the anesthetic tank. After the procedure, the fish quickly recovers from anesthesia by placing in a recovery tank, or by flushing the gills with water.

Frogs
As with fish, MS-222 should always be buffered with sodium bicarbonate to a pH of 7.0-7.5 prior to using with animals.
Surgical anesthesia in adult frogs: 1 to 2 g/liter
Tadpoles: 0.2 to 0.5 g/liter

The frog is immersed in the buffered solution (pH 7.0-7.5); a surgical plane of anesthesia is reached within 10-15 minutes. The animal can then be removed and anesthesia maintained by dripping solution onto the skin. After the procedure, the frog can be recovered from anesthesia by rinsing in clean, dechlorinated water and placing into a recovery tank.

EUTHANASIA

Fish
The fish is immersed in an overdose concentration of MS-222. Concentrations exceeding 300-400 mg/liter constitute overdoses for most fish. Adult Zebrafish should be euthanized by rapid cooling. Solutions should still be buffered to prevent irritation. Fish should remain in solution 10 minutes following cessation of operculum movements.

Frogs
The frog is immersed in an overdose concentration of MS-222. Concentrations exceeding 3 gm/liter constitute overdoses for most species. Solutions should still be buffered to prevent irritation. Prolonged immersion (as long as 1 hour) may be required for 5 to 10 g/L water baths. This must be followed by an adjunctive method of euthanasia; either pithing or decapitation. Decapitation can be performed with heavy shears or a guillotine, and must be followed by pithing or another method of destroying brain tissue. The pithing site in frogs is the foramen
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magnum, which can be identified by a slight midline skin depression posterior to the skull, midline between the eyes, with the neck flexed.

DISPOSAL OF EXPIRED AND PREPARED MS-222

Expired MS-222: should be disposed of by contacting the Department of Environmental Health and Radiation Safety (EHRS) of the University of Pennsylvania.

Used or prepared solutions of MS-222: should be collected in approved containers (1 or 5 gallon) from EHRS. EHRS can be contacted to collect containers when they are ready for disposal. Do not discard MS-222 directly into drain systems, surface water, storm water conveyances or catch basins.

Please contact the Department of Environmental Health and Radiation Safety to request a chemical waste pickup and order supplies using their online form at www.ehrs.upenn.edu/chemwaste

If you have additional questions about the use of MS-222, please contact the ULAR veterinarian.

PHARMACEUTICAL GRADE SOURCES

Western Chemical’s TRICAINE-S (MS-222, TMS, tricaine methanesulfonate) is an FDA-approved fish anesthetic (FDA ANADA 200-226).

REFERENCES

1. American Veterinary Medical Association Guidelines for Euthanasia (2013) (AVMA)
2. Guide for the Care and Use of Laboratory Animals (Guide)