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Title: Tricaine Methanesulfonate (MS-222) Preparation and Use

I. Purpose:

Tricaine Methanesulfonate (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. 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. This document provides guidance on the accepted method of preparation and use of the substance.

II. Policy:

Uses:

Tricaine Methanesulfonate is appropriate for the temporary immobilization of fish, amphibians, and other aquatic, cold-blooded animals.

Pharmaceutical grade MS-222 must be used in a **buffered** solution for all intended uses on live vertebrate aquatic species.

III. Procedure:

The 2013 American Veterinarian Medical Association (AVMA) Guidelines for the Euthanasia of Animals states:

"A 10 g/L of stock solution can made and sodium bicarbonate added to saturation resulting in a pH of 7.0 to 7.5 for the solution."

The stock solution should be stored in a dark brown bottle, and refrigerated or frozen if possible. The solution should be replaced monthly and any time a brown color is observed.

"MS-222 is an acceptable method of euthanasia for finfish and for some amphibians and reptiles. When used for large finfish and some amphibians (eg, Xenopus spp.), a secondary method should be used to insure death."

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Below are two examples of Pharmaceutical Grade sources available to use as a buffered solution.

1. FINQUEL MS-222 - 99.5% Pure Tricaine Methanesulfonate
2. Western Chemical's TRICAINE-S (MS-222, TMS, tricaine methanesulfonate) is an FDA approved fish anesthetic (FDA ANADA 200-226)

DIRECTIONS FOR USE ON FISH CONCENTRATIONS

MS-222 is effective and safe for the anesthesia of fish when used as directed. Its use is governed by, and can be tailored to, the needs of individual fishery personnel. Sedation and various rates of anesthetization are controlled by the concentration. The versatility of the two sources is demonstrated by the fact that it has been used in fisheries at levels ranging from 10 to 1,000 mg./liter. The action of the anesthetic is slowed at cooler temperatures, in extremely soft water (approximately 10 mg./liter of CaCO₃ or less), and in larger fish. Also, efficacy may vary with species. Thus, it is imperative that preliminary tests of anesthetic solution be made against small numbers of fish to determine the desired rates of anesthesia and exposure times for the specific lots of fish under prevailing conditions.

METHODS OF APPLICATION

1. General anesthesia: - For most situations where rapid or moderately rapid anesthesia is required, MS-222 may be applied in a bath, i.e., the fish are immersed in the anesthetic solution. Containers may be of glass, plastic, steel, aluminum, or other suitable material. However, do not use galvanized or brass containers unless treated or sealed to prevent dissolution of zinc. Size of container is determined by individual needs, but the fish should not be overcrowded. Discard anesthetic solutions when a loss in potency is noted, or when the solutions become fouled with mucus or excrement.

2. For surgery and certain physiologic studies, the fish may be anesthetized to loss of reflex, removed from the anesthetic, and then positioned so that the gills are bathed in a sedating concentration of MS-222. Some investigations have developed flowing, recirculating systems for bathing the gills with anesthetic during surgery.

Large fishes such as sharks and rays are anesthetized within minutes by spraying the gills with a 1 g./liter solution of MS-222. The application is made by means of a water pistol, bulb syringe, hand pump, etc.

3. Transport – MS-222 has been used to sedate fish during transport. It is more successful in cold than in warm water, and it is instrumental in reducing injuries because of hyperactivity. Fish are usually transported by means of distribution units (tank trucks), or by air in plastic bags. In either case, the fish should be fasted before-hand to reduce metabolic wastes. Also, some workers suggest pre-transport sedation for several hours to lower metabolism. With distribution units, the fish may be fasted and sedated prior to loading. The anesthetic solution is prepared in the distribution unit and oxygenated. Then, the fish are added and temperature acclimated.

4. Dosage - Use: Western Chemical Inc. has incorporated a working spreadsheet on their website to help with determining dosages.

The American Fisheries Society - Fish Culture Section's Working Group on Aquaculture Drugs, Chemicals, and Biologics (WGADCB) has published a Guide to Using Drugs, Biologics and Other Chemicals in Aquaculture. WGADCB have also developed a Treatment Calculator to assist in the dosage calculations of certain products.

The Guide is provided as an Adobe PDF file and the Treatment Calculator is offered as a Microsoft Excel 2007 file. Your computer must have installed either Microsoft Excel or any other program that will read and execute calculations of an Excel file.

The Guide and Treatment Calculator can be found by clicking the link below

[Guide to Using Drugs, Biologics and Other Chemicals in Aquaculture](#)

Summary of Storage and Expiration dates:

MS-222 powder = See expiration date on bottle

Working solution = 4 weeks from the date of preparation when stored at 4°C or when degradation of solution has been seen. (i.e. brown color occurs)

Concentrated Solutions = should be stored at -20°C, and should list the expiration date of the powder from the parent bottle.

5. Safety:

MS-222 Disposal:

Must be disposed of through EH&S hazardous disposal services or another acceptable method.

The waste water treatment plant does not want this product in the water system. Email the UCD Hazardous Waste Office at hazwaste@ucdavis.edu with any questions.

The price for disposal of pure product would be \$10/lb. and if it is in water the price is \$20/gal.

For a MSDS please view Argent Chemical Laboratories for Finquel® at this link:

<http://www.ucmsds.com/?X>

Some good examples of safe practices when working with MS-222 (tricaine methane sulphonate):

- Wear protective clothing, gloves and goggles when handling the MS-222 powder.
- If possible, 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. If Engineering Controls are unavailable, use appropriate PPE to work with the powder safely.

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- 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.
- Do not discard MS-222 directly into surface water, storm water conveyances or catch basins.

References:

American Veterinary Medical Association Guidelines for the Euthanasia of Animals:2013 Edition;
<https://www.avma.org/KB/Policies/Documents/euthanasia.pdf>

Laboratory Animals: Recommendations for euthanasia of experimental animals. Royal Society of Medicine Press, London, U.K. 1996.

Evaluation and Refinement of Euthanasia Methods for *Xenopus laevis*. JAVMA. Vol. 48, No. 5, September 2009. Pages 512-516

Argent Chemical Labs - <http://www.argent-labs.com/>

Western Chemical Inc. -<http://www.wchemical.com/TRICAINE-S-MS-222-P43C7.aspx>

<http://www.drugs.com/vet/finquel-ms-222-tricaine-methanesulfonate.html>