INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

Policy #13: Tricaine Methanesulfonate (MS-222) Preparation, Storage and Use
Approved: New

A. Purpose
This document provides accepted methods of preparation, storage and use of MS222.

B. Background
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, coldblooded animals. It is also approved by AVMA as an acceptable method of euthanasia for finfish and for some amphibians and reptiles. There are safety precautions required when working with the concentrated powder and stability/storage issues once made into solution, which are describe in detail below.

C. Policy
Tricaine Methanesulfonate is appropriate for the temporary immobilization of fish, amphibians, and other aquatic, cold-blooded animals. MS-222 is an acceptable method of euthanasia for finfish and for some amphibians and reptiles. When used for large finfish and some amphibians (e.g., Xenopus spp.), a secondary method should be used to ensure death. For more information, please refer to WSU IACUC Policy #28 regarding euthanasia of poikilothermic vertebrates.

Pharmaceutical grade MS-222 must be used in a buffered solution for all intended uses on live vertebrate aquatic species. Currently Tricaine-S (a USFDA registered drug of Western Chemical Inc.) is the only available FDA approved MS-222 product on the market.

D. Preparation
Concentrated MS-222: To avoid inhalation, use in a well ventilated area when working with the concentrated powder. Engineering controls to limit inhalation of fine crystalline powder is advised, including use of MS-222 powder within a class II biosafety cabinet or a fume hood. To avoid skin contact, goggles, gloves, and a lab coat are advised. Individuals working with MS-222 should be familiar with the MSDS available at https://smhttp-ssl-29106.nexcesscdn.net/media/docs/tricaine_s_sds.pdf. See Appendix A for the WSU Chemical Standard Operating Procedure

MS-222 is water soluble and should be prepared in water similar to the culture conditions of the animal. The water should have adequate levels of dissolved oxygen, and appropriate pH, temperature, alkalinity, hardness and salinity for the subjects. MS-222 can acidify aqueous solution therefore the solution should be buffered (using sodium bicarbonate) to a neutral pH (approximately 7.0) appropriate for the species before use.
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. A dosage chart is available from the manufacturer at https://smhttp-ssl-29106.nexcesscdn.net/media/docs/tricaine_s_dosage_chart.pdf

For euthanasia, fish need to be immersed in concentrated MS-222 water and need to be kept in the solution for at least 10 min following cessation of opercular movement. A concentration of at least 250mg/L is recommended and might be much higher in certain species. In large fish, the animal might also be removed from the holding tank and a concentrated MS222 solution flushed over the gills until death is confirmed or until deeply anesthetized and followed by a secondary method of euthanasia (e.g. pithing, cervical transection, captive bolt or rapid freezing).

Additional recommendation for the preparation and use of Tricaine-S are online at https://smhttp-ssl-29106.nexcesscdn.net/media/docs/tricaine_s_directions_for_use.pdf.

E. Storage
Use of freshly prepared solutions is recommended, as preparations 10 days old had a 5% decreased potency at room temperature. Non-buffered stock solution can be stored in the refrigerator for up to 30 days. Non-buffered concentrated stock solutions can be stored at -20°C, and should list the expiration date of the powder from the parent bottle. MS222 is light sensitive so should be stored in an opaque air-tight container. The solution must be replaced any time a brown color is observed in the liquid.

F. References

WSU-IACUC Policy #13: Approved 10.31.2018
### PROCEDURE / PROCESS

MS-222 is used for anesthesia and euthanasia of fishes and other aquatic species. MS-222 is water soluble and should be prepared in water similar to the culture conditions of the animal. The water should have adequate levels of dissolved oxygen, and appropriate pH, temperature, alkalinity, hardness and salinity for the subjects. The prepared solution should be buffered to a neutral pH (approximately 7.0) before use. Use of freshly prepared solutions is recommended, as preparations 10 days old had a 5% decreased potency.

**Use as an anesthetic:** 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. Preliminary tests are necessary to determine the concentration and exposure time for each application. Higher concentrations of MS-222 result in rapid anesthesia with shorter maximum tolerated exposure times. Commonly used concentrations for rapid anesthesia range from 70 – 330 mg/L. Lower concentrations of MS-222 result in longer induction times and longer maximum tolerated exposure time. Commonly used concentrations for moderately rapid anesthesia range from 50 – 70 mg/L. Animals are revived by returning them to clean, untreated water preferably from their home environment.

**Use for euthanasia:** MS-222 can be used to euthanize fish. Preliminary tests are necessary to determine the concentration (mg/L) and exposure time necessary for mortality. The concentration of MS-222 used for euthanasia should result in medullary collapse (opercular activity ceases in fish). The exposure time should be adequate that a return to fresh water will not result in recovery of the animal.

### CHEMICAL NAME(S) and associated PHYSICAL and HEALTH HAZARDS

**MS-222; CAS No: 886-86-2**

MS-222 is an acidic solution and may be irritating by inhalation, ingestion or absorption through the skin.

### LABELING

Containers with MS-222 shall have labels identifying contents, hazards, precautionary measures, and emergency contact information. Animals treated with MS-222 shall be identified with signs or cards on the tanks which have the appropriate hazard warning symbol (see above) and/or state: “Caution: animals treated with MS-222”.

### PROTECTIVE EQUIPMENT

**Concentrated MS-222:** To avoid inhalation, use in a well ventilated area for most applications. For large volumes, engineering controls to limit inhalation of fine crystalline powder is advised, including use of MS-222 powder within a class II biosafety cabinet or a fume hood. To avoid skin contact, goggles, gloves, and a lab coat are advised.

**MS-222 in water (tanks):** HHS has determined under 21 CFR 25.33(a)(1) that water treated with MS-222 does not individually or cumulatively have a significant effect on the human environment.
| WASTE DISPOSAL PROCEDURES | **Concentrated MS-222**: Waste must be in an airtight compatible container, labeled with a completed Dangerous Waste label, accompanied with completed Chemical Collection Request Form, and held until picked up by EH&S.  
**Water treated with MS-222**: If pH is between 6–10, dispose of into sanitary sewer. Contact EHS for hazardous waste disposal beyond this pH range. |
| DECONTAMINATION PROCEDURES | **Upon Accidental Exposure**: Eye or skin contact: flush eyes or skin with copious amounts of water; Accidental inhalation or ingestion: immediately seek medical attention and follow instructions on MSDS.  
**Upon Accidental Release**: If significant amounts of concentrated MS-222 are released outside engineered controls, immediately evacuate and secure area and contact EH&S. If a small amount of concentrated MS-222 is released, wear chemical resistant gloves and chemical splash goggles, spray spill with water to limit airborne exposures, absorb and remove to appropriate containers, and dispose of as hazardous waste (see above WASTE DISPOSAL PROCEDURES). |