A. Purpose

This document provides accepted methods of preparation, storage and use of Tricaine Methanesulfonate (MS-222).

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 (eg, 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 [here](#). 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 [here](#).

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 recommendations for the preparation and use of Tricaine-S are available [online](#).
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