ANIMAL EYE CONSULTANTS OF IOWA

Animal Eye Consultants of Iowa

Institutional Animal Care and Use Committee

Policy #13	**"Tricaine Methanesulfonate (MS-222) Preparation, Storage and Use"**
Approval Date: 11/28/2023 (Replacing Version 12/9/2020)	

A. Purpose

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

B. Background

MS-222 is a popular anesthetic agent intended for the temporary immobilization of fish, amphibians, and other aquatic poikilotherms. It is also approved by the AVMA as an acceptable method of euthanasia for finfish, 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. MS-222 is not FDA-approved for animals processed for human consumption.

C. Policy

MS-222 is appropriate for the temporary immobilization of fish, amphibians, other aquatic poikilotherms. MS-222 is an acceptable method of euthanasia for finfish, 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 AEC 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, work with the concentrated powder, which is fine and crystalline, in a well-ventilated area, 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 and the AEC Chemical Standard Operating Procedure.

MS-222 is water soluble and should be prepared in water that is similar to the water 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 may acidify aqueous solution. Therefore, the solution should be buffered with 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 the concentration and exposure time for each application to ensure sufficient anesthetic depth and safe recovery. A dosage chart is available from the manufacturer here.

For **euthanasia,** fish must be immersed in a concentrated MS-222 solution for at least 30 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 MS-222 solution flushed over the gills until death is confirmed or until deeply anesthetized, 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 here.

E. Storage

Use of freshly prepared solutions is recommended, because 10-day old preparations show a 5% decrease in potency after storage 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. MS-222 is light sensitive and therefore 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. Exposure Emergency

SKIN, EYE OR MUCOUS MEMBRANE EXPOSURE TO TREATED WATER

1. Flush immediately at nearest eyewash station or emergency shower for 15 minutes.

2. Notify your supervisor.

3. Seek care: Take MS-222 safety data sheet (SDS) with you.

4. Report Incident: For all incidents and near misses, the involved person or supervisor completes and submits the AEC HRS Incident Report form within 24 hours.

G. Waste Disposal

Indoor Applications:

- **Solid MS-222** waste (powder) and stock solutions need to be collected by EH&S as *regulated* chemical waste. To request pickup of solid waste, visit the EH&S Chemical Waste Disposal website.
- **Liquid MS-222** waste, such as tank water, can be disposed of via sanitary sewer if the liquid solution contains less than a 10% concentration of MS-222. MS-222 waste containing more than a 10% concentration needs to be collected by EH&S as regulated chemical waste.

Note that MS-222 solutions react with bleach and other disinfectants, creating a sticky, somewhat waxy yellow to orange precipitate that can be difficult to remove from containers.

H. References

1. Bowker, J.D., J.T. Trushenski, Editors. 2016. Guide to Using Drugs, Biologics, and Other Chemicals in Aquaculture. American Fisheries Society Fish Culture Section.
2. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition
3. Harper, C., Lawrence C., “The Laboratory Zebrafish” CCR Press 2011
4. Green, S. L. (2010). *The Laboratory Xenopus sp.* Taylor and Francis Group, LLC.
5. https://syndel.com/wp-content/uploads/2019/01/tricaine_s_sds.p