WVU IACUC Policy:
Preparation and Use of Tricaine Methane Sulfonate (TMS, MS-222)

Background

MS-222 is a widely used anesthetic and euthanasia agent for fish and amphibians. This document provides safety recommendations for product preparation, compound recommendations, and doses.

Purpose

To provide guidance on the use of MS-222 in animal studies and to provide standardized methods for its preparation and storage.

Responsibilities

The Principal Investigator (PI) ***must*** ensure that all staff are trained in the proper use and preparation of this compound.

The PI ***must*** ensure the proper formulation is purchased and used.

If administering to food animal species (including aquatic wildlife or aquaculture) appropriate withdrawal times and pharmaceutical label indications ***must*** be adhered to. Syncaine® label states the following: **Do not use within 21 days of harvesting fish for food. Use in fish intended for food should be restricted to Ictaluridae, Salmonidae, Esocidae, and Percidae, and water temperature exceeding 10°C (50°F). In other fish and in cold-blooded animals, SYNCAINE should be limited to hatchery or laboratory use. Avoid inhaling or getting into eyes.**

Off label use will cause animals to be considered adulterated and unfit for consumption.

Associated WVU IACUC Policies & Guidelines

- Veterinary Recommendations for Anesthesia and Analgesia
- Non-Pharmaceutical-Grade Substances Used in Animals (*if applicable*)

Considerations

- **Formulation**
  - The IACUC strongly recommends using a pharmaceutical-grade MS-222 product (e.g. Syncaine® manufactured by Syndel). This product ***must be buffered*** to a neutral pH prior to use for all species.
  - Non-pharmaceutical-grade formulations ***must*** be scientifically justified and source clearly described in the IACUC protocol.

- **Safety Precautions**
    - MS-222 is an irritant to the eyes, respiratory tract and skin. Safety measures ***must*** be utilized when working with this compound.
    - To prevent exposure, the powder should be weighed in a chemical fume hood, Class II Biosafety Cabinet (BSC) or by wearing an N95 respirator. To avoid skin and eye contact, goggles, gloves and lab coat/protective clothing should be worn.
    - Personnel who are required to wear respirators ***must*** be cleared through WVU Occupational Medicine, to ensure proper fitting masks and testing.
    - When handling liquid containing MS-222, personal protective equipment (PPE) should include gloves, lab coat and eye goggles to prevent contact with eyes or skin.
    - If performing field work, ensure that you are working with the compound in a well-ventilated area (outdoors) and are wearing the proper PPE described above.

Preparation

- MS-222 comes in a powdered form and should be stored at room temperature, in a cool dry place protected from light.
- 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.
- The solution ***must*** be buffered with sodium bicarbonate to a neutral pH (approximately 7.0) appropriate for the species, before use.
- MS-222 buffered stock solutions should be utilized the same day as preparation per vendor's recommendation or stored appropriately for future use. Solutions ***must*** be stored in tinted (amber) or opaque bottles.
    - The stock solution has been shown to be stable at 4ºC or -20ºC for up to 6 months. The solution should be replaced when the powder expires, 6 months after reconstitution, and any time a brown color is observed.
    - Before using, the temperature of the solution should match the temperature the animals are currently at.

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. Pilot tests are recommended.
- The dose, route and frequency should be specified in the IACUC protocol.
- After procedures are completed, place anesthetized fish in well-oxygenated/aerated, un-medicated water.
- Closely monitor fish recovering from anesthesia until they are swimming normally and have completely regained their righting response.

Use for Euthanasia

- MS-222 is considered an acceptable method per the AVMA Guidelines on Euthanasia.
- Immerse fish in buffered MS-222 for at least 30 minutes; it is recommended that this is followed by an adjunctive method (decapitation, pithing, exsanguination, freezing).
- A concentration of 250-500 mg/L or 5 to 10 times the anesthetic dose is effective for most species.
- The exposure time should be adequate so that a return to fresh water will not result in recovery of the animal. Due to species differences in response to MS-222, a secondary method of euthanasia is recommended in some finfish and amphibians to ensure death. Regardless of whether a secondary method is used, death ***must*** be confirmed prior to carcass disposal.

Manufacturer Dosage Chart

Tricaine-S Dosage Chart

Grams of Tricaine-S (TMS, MS-222) required for various dosages in various container sizes

	Anesthetic Container Size (Liters)										
Dosage (mg/L)	1	3.78 (1 Gal)	5	10	20	30	40	50	100	500	1000
30	0.03 g	0.11 g	0.15 g	0.30 g	0.60 g	0.90 g	1.20 g	1.50 g	3.00 g	15.00 g	30.00 g
35	0.04 g	0.13 g	0.18 g	0.35 g	0.70 g	1.05 g	1.40 g	1.75 g	3.50 g	17.50 g	35.00 g
40	0.04 g	0.15 g	0.20 g	0.40 g	0.80 g	1.20 g	1.60 g	2.00 g	4.00 g	20.00 g	40.00 g
45	0.05 g	0.17 g	0.23 g	0.45 g	0.90 g	1.35 g	1.80 g	2.25 g	4.50 g	22.50 g	45.00 g
50	0.05 g	0.19 g	0.25 g	0.50 g	1.00 g	1.50 g	2.00 g	2.50 g	5.00 g	25.00 g	50.00 g
55	0.06 g	0.21 g	0.28 g	0.55 g	1.10 g	1.65 g	2.20 g	2.75 g	5.50 g	27.50 g	55.00 g
60	0.06 g	0.23 g	0.30 g	0.60 g	1.20 g	1.80 g	2.40 g	3.00 g	6.00 g	30.00 g	60.00 g
65	0.07 g	0.25 g	0.33 g	0.65 g	1.30 g	1.95 g	2.60 g	3.25 g	6.50 g	32.50 g	65.00 g
70	0.07 g	0.26 g	0.35 g	0.70 g	1.40 g	2.10 g	2.80 g	3.50 g	7.00 g	35.00 g	70.00 g
75	0.08 g	0.28 g	0.38 g	0.75 g	1.50 g	2.25 g	3.00 g	3.75 g	7.50 g	37.50 g	75.00 g
80	0.08 g	0.30 g	0.40 g	0.80 g	1.60 g	2.40 g	3.20 g	4.00 g	8.00 g	40.00 g	80.00 g
85	0.09 g	0.32 g	0.43 g	0.85 g	1.70 g	2.55 g	3.40 g	4.25 g	8.50 g	42.50 g	85.00 g
90	0.09 g	0.34 g	0.45 g	0.90 g	1.80 g	2.70 g	3.60 g	4.50 g	9.00 g	45.00 g	90.00 g
95	0.10 g	0.36 g	0.48 g	0.95 g	1.90 g	2.85 g	3.80 g	4.75 g	9.50 g	47.50 g	95.00 g
100	0.10 g	0.38 g	0.50 g	1.00 g	2.00 g	3.00 g	4.00 g	5.00 g	10.00 g	50.00 g	100.00 g

Disclaimer* Western Chemical Inc. or Syndel Laboratories Ltd. do not guarantee the appropriateness or accuracy of any calculation made using this table as these estimated doses are provided for information purposes only. Users are encouraged to carefully calculate their own anesthetic bath volumes and associated dosages. Always follow label directions and test on a small group of fish prior to use. If you are unfamiliar with using this product, we recommend that you first consult with your local fish health professional before using this product.

The *AQUATIC LIFE SCIENCES* Companies

Syndel Laboratories, Ltd.
Available in Canada from Syndel Laboratories Ltd (800) 663-2282 www.syndel.com

Western Chemical, Inc.
Available in the USA from Western Chemical Inc. (800) 283-5292 www.wchemical.com

Exposure Emergency

- Flush immediately at nearest eyewash station or emergency shower for 15 minutes.
- Notify your supervisor.
- Seek care; take MS-222 safety data sheet (SDS) with you.

Waste Guidance

- Contact EH&S for a waste determination.
- If working in the field, all waste ***must*** be disposed of properly at the University and not discarded in the field.
- Solid MS-222 waste (powder) and stock solutions need to be collected by EH&S as regulated chemical waste.
- Liquid MS-222 waste, such as tank water, may need to be disposed of as regulated waste depending on concentration.
- Carcasses cannot be left in the field due to concerns of scavenging and environmental contamination. They ***must*** be properly disposed of based on EH&S guidance.

References

Michigan State University. *MS-222 Safety Guideline*. Environmental Health & Safety, https://ehs.msu.edu/_assets/docs/chemical/ms-222-safety-guideline.pdf..

Syndel. *Syncaine CDN Safety Data Sheet (Rev. 03Dec2021)*. 3 Dec. 2021, https://syndel.com/wp-content/uploads/2021/12/Syncaine-CDN-SDS-03Dec2021-Rev.03Dec2021.pdf..

University of Georgia. *MS-222 Guidelines*. Office of Animal Care and Use, https://research.uga.edu/docs/policies/compliance/oacu/UGA-IACUC-MS222-Guidelines.pdf.

University of Toledo Institutional Animal Care and Use Committee (IACUC) Guidelines for the Use of Tricaine Methanesulfonate (MS-222) in Fish, https://www.utoledo.edu/research/rsp/RC/animal/docs/tricaine-methanesulfonate-guideline.pdf

Washington State University Tricaine Methanesulfonate (MS-222) Preparation, Storage and Use, https://iacuc.wsu.edu/documents/2018/11/policy-13.pdf/

University of California, Davis Tricaine Methanesulfonate (MS-222) Preparation and Use, https://research.ucdavis.edu/wp-content/uploads/SC-40-406.pdf