



IACUC Guidance:	TAMU-G-021	Title:	Guidelines for Preparing MS222	
	Location	Effective Date	Review By	
	College Station/Dallas/Galveston/Kingsville	06/01/2024	05/31/2027	
	Houston	N/A	N/A	

1. PURPOSE

- 1.1. This document provides guidance regarding safety for personnel preparing MS222 solutions (Tricaine Methanesulfonate or (“Tricaine-S”), the proper usage of the compound, common dosages for different applications and proper disposal.

2. SCOPE

- 2.1. Applies to use of MS222 in/on animals used for research, teaching, testing or other purposes at Texas A&M University. This document is intended to augment, but not supersede, safety and handling information provided on the manufacturer’s drug label or provided by EHS.
- 2.2. See TAMU-G-002 for more information on anesthetics and their use, including record requirements.
- 2.3. See TAMU-G-008 for Guidelines on Working with Zebrafish.
- 2.4. See TAMU-G-048 for Guidelines on Euthanasia of fish, Amphibians and Reptiles.
- 2.5. See TAMU-G-049 for Guidelines on Performing Surgery in Amphibians and Reptiles.

3. RESPONSIBILITY

- 3.1. It is the responsibility of the **PI** to assure the safe use of MS222 by laboratory staff.
- 3.2. **Personnel** using MS222 should be familiar with the SDS, which should be readily available in the lab.

4. DEFINITIONS AND/OR ACRONYMS

- 4.1. **AV:** Attending Veterinarian. Individual designated by Texas A&M University to fulfil the regulatory role of AV. May also describe veterinary staff who report directly to, and have delegated authority from, the AV.
- 4.2. **CMP:** Comparative Medicine Program
- 4.3. **EHS:** Environmental Health and Safety Department
- 4.4. **Extra-Label:** Use of an FDA-approved drug in a way that is not described on the drug’s labeling. May also be referred to as “off-label”.
- 4.5. **FDA:** U.S. Food and Drug Administration
- 4.6. **SDS:** Safety Data Sheet. Document that provides information relating to occupational health and safety for the use of various drugs, chemicals, chemical compounds, or chemical mixtures and is a component of the Globally Harmonized System of Classification and Labeling of Chemicals.
- 4.7. **MS222:** Tricaine Methanesulfonate is an FDA-approved drug (“Tricaine-S”) for temporary immobilization (sedation, anesthesia) of finfish, amphibians, and other aquatic, cold-blooded animals.

5. GUIDELINES OR PROCEDURE

5.1. General

- 5.1.1. Used for the temporary immobilization of fish, amphibians, and other aquatic, cold-blooded animals.
- 5.1.2. Administered as a solution, by immersion. When used for induction and maintenance of anesthesia, the concentration used determines the depth of anesthesia. The anesthetic solution should be buffered before use, using sodium bicarbonate or seawater.
- 5.1.3. MS222 is not FDA approved for use as an agent of euthanasia. Described as an extra-label euthanasia agent in fish, some amphibians, and reptiles in the AVMA Guidelines for the Euthanasia of Animals, 2020 Edition.
- 5.1.4. MS222 has a 21-day withdrawal period for fish. Fish exposed to MS222 may not be used as food sources for humans or other animals. Withdrawal period may impact releasing fish back to the wild. PI must adhere to release restrictions described on the applicable state, federal or international permit associated with the AUP.

5.2. Safety

- 5.2.1. MS222 is an irritant to the eyes, respiratory system, and skin.
- 5.2.2. MS222 powder should be weighed under a fume hood or wearing an N95 mask in a well-ventilated area.
- 5.2.3. Goggles, gloves and a lab coat/protective clothing should be worn while measuring the powder and handling solutions.
 - 5.2.3.1. Nitrile gloves are recommended.
- 5.2.4. Use a top loading balance with a clear plastic wind/breeze guard to minimize dispersion of the powder.
- 5.2.5. Minimize contact with human skin, particularly when dissolved.

5.3. Preparation

- 5.3.1. MS222 comes in a powdered form and should be stored at room temperature, in a cool dry place protected from light.
- 5.3.2. The use of non-pharmaceutical grade MS222 requires IACUC approval. See TAMU-G-010.
- 5.3.3. Diluents or vehicles must be specified in the animal use protocol.
- 5.3.4. Sterile water/sterile saline and aseptic technique must be used for preparation of MS222 for injections.
 - 5.3.4.1. The product must be passed through a syringe filter (0.22 μm or finer). This can be done in the process of transfer to a sterile injection vial.
 - 5.3.4.2. MS222 for injection has to be prepared fresh each time.
- 5.3.5. Dry MS222 and dry sodium bicarbonate should be stored in separate containers.
- 5.3.6. A stock solution can be prepared for use in water bath or spray applications. Commonly used MS222 stock solutions are at 10g/L.
 - 5.3.6.1. A 10g/L stock solution can be made and discarded after 30 days, or before the expiration date of the powder from the parent bottle, whichever comes first.
 - 5.3.6.2. Must be labeled with the agent, concentration, date of preparation and date of expiry.
 - 5.3.6.3. Must be protected from light (amber glass bottle and/or wrapped in tin foil).
 - 5.3.6.4. Stored in freezer at 4°C.
 - 5.3.6.5. Discarded if any degradation is seen (i.e., oily film or discoloration of solution).
- 5.3.7. All working solutions **must** be buffered to a pH of 7.0-7.5 (or the ambient environmental pH of the study species), by the addition of sodium bicarbonate (NaHCO_3), or seawater.
 - 5.3.7.1. Since unbuffered MS222 is so irritating to amphibian skin, several buffering methods for have been described:
 - 5.3.7.1.1. The pH of a 1 g/L MS222 solution can be raised from 3.0 to 7.0 by addition of 34 mL of 0.5 M Na_2HPO_4 to 2 L of the 1 g/L MS-222 solution.
 - 5.3.7.2. Sodium bicarbonate can also be used as a buffer, at 420–1,050 mg/L (10–25 mEq/L).
 - 5.3.7.2. The pH of the solution must be checked prior to use with either a calibrated pH meter or pH paper to ensure neutrality.
 - 5.3.7.3. Water should be taken from the animal's original holding tank or natural water source.
 - 5.3.7.4. Working solutions should not be re-used nor should they be stored for future use.

5.4. Use as an Anesthetic Agent

- 5.4.1. Preliminary Testing
 - 5.4.1.1. Determines the concentration and exposure time for each application to assure sufficient anesthetic depth and safe recovery.
 - 5.4.1.2. The action of MS222 as an anesthetic varies widely between species and is affected by water temperature, hardness, and size/age of the individual animal.
 - 5.4.1.3. Contact the AV or designee for additional information.
- 5.4.2. Induction of Anesthesia:
 - 5.4.2.1. Use higher concentrations of MS222 for rapid onset of anesthesia with shorter maximum tolerated exposure times.
- 5.4.3. Maintenance of Anesthesia:
 - 5.4.3.1. Use lower concentrations of MS222.
 - 5.4.3.2. Allow animal to reach appropriate level of anesthesia prior to initiation of approved procedure.

- 5.4.3.3. For more details regarding anesthetic monitoring, see TAMU-G-035 and TAMU-G-049.
- 5.4.4. Maintain adequate oxygenation of the animal throughout induction, anesthesia, and recovery.
 - 5.4.4.1. Oxygenation of the water can be accomplished by supplying pure oxygen via diffusers or similar devices.
 - 5.4.4.2. Aeration can be accomplished by supplying clean air via an air pump and air stone, or similar devices.
 - 5.4.4.3. Continuous delivery of anesthetic (and oxygen) in water to the gills of fish is necessary for long surgical procedures.
- 5.4.5. Dosages for MS222 solutions (i.e., water baths) are sometimes provided in different units such ratio, parts per million and percentage weigh/volume.
 - 5.4.5.1. Examples for those dosages as per mg/L, ratio [®], parts per million (ppm), percentage weight/volume (%w/v) and percentage % are:
 - 5.4.5.1.1. 50 mg/L = 1:20,000 [®] = 50 ppm = 0.005 % w/v
 - 5.4.5.1.2. 100 mg/L = 1:10,000 [®] = 100 ppm = 0.01 % w/v
 - 5.4.5.1.3. 1 g/L = 1: 1,000 [®] = 1000 ppm = 0.1 % w/v

5.4.6. Dosages for Anesthesia

- 5.3.1.1. Consult with the AV, or designee for more information.
- 5.3.1.2. Table of common dosages:

Taxon	Dosage	Comments
Fish	15-50 mg/L	Sedation
	50-200 ⁺ mg/L	Induction
	50-100 [#] mg/L	Maintenance
	1 g/L	Spray – large fish on gills directly
Amphibians	50-200 mg/kg	Most species – intracoelomic injection
Frogs/Salamander	0.5-2 g/L	Bath to effect/induction in 15-30 min
Leopard Frogs	100-200 mg/L	Intracoelomic injection
Bullfrogs	100-400 mg/L	Intracoelomic injection

⁺100-200 mg/L for zebrafish

[#]40-100 mg/L for zebrafish

* Higher levels of MS222, such as 200-300mg/L, are sometimes used for non-survival procedures

5.5. Use of MS222 for euthanasia

- 5.5.1. Used for euthanasia of reptiles, amphibians, and fish. See TAMU-G-048.
- 5.5.2. There appears to be a substantial species-specific response to MS222.
 - 5.5.2.1. Preliminary tests are necessary to determine the concentration (mg/L) and exposure time necessary for mortality.
 - 5.5.2.2. Some species requiring higher doses or secondary measures to ensure death; please consult with the AV, or designee during protocol development.
- 5.5.3. Potency of MS222 is increased in warm water and decreased in cold water.
- 5.5.4. The concentration of MS222 used for euthanasia should result in rapid loss of consciousness (loss of righting response, cessation of rhythmic opercular activity).
- 5.5.5. Ensure adequate exposure time.

5.6. Disposal

- 5.6.1. Disposal of MS222 solutions must occur in accordance with recommendations by EHS.

6. RECORDS

- 6.1. Research records, including records of drug administration, must be maintained consistent with Texas A&M University Standard Administrative Procedures (SAPS) 15.99.03.M1.03. and 29.01.03.M0.01

7. EXCEPTIONS

- 7.1. The PI may request an exception to the above standards by describing the departure in the AUP



- 7.2. For programmatic exceptions, the facility director or manager may submit a request for the exception using TAMU-F-013

8. REFERENCES, MATERIALS, AND/OR ADDITIONAL INFORMATION

8.1. References

- 8.1.1. American Veterinary Medical Association. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition.
- 8.1.2. Bowker, J.D., J.T. Trushenski, M.P. Gaikowski, and D.L. Straus, Editors. 2012. Guide to Using Drugs, Biologics, and Other Chemicals in Aquaculture. American Fisheries Society Fish Culture Section.
- 8.1.3. Brown University Institutional Animal Care and Use Committee Guidelines on the use of Tricaine-S (MS-222) Page 3 of 3
- 8.1.4. Carpenter, JW, CJ Marion, eds. (2013): Exotic Animal Formulary, 4th Edition. Elsevier Saunders, St. Louis.
- 8.1.5. CITI:
Web page: <https://about.citiprogram.org/en/homepage/>
Instructions: <https://rcb.tamu.edu/animals/training>
Courses:
CITI Working with Zebrafish (Danio rerio) in Research Settings
CITI Working with Fish in Research Settings
- 8.1.6. Convoy, CJ, T Papenfuss, J Parker, NE Hahn (2009): Use of Tricaine Methanesulfonate (MS222) for Euthanasia of Reptiles. J Am Assoc Lab Anim Sci. 2009 January; 48(1): 28-32.
- 8.1.7. Fernandes, IM et al. The efficacy of clove oil as an anaesthetic and in euthanasia procedure for small-sized tropical fishes. Braz J Biol. 2017 Jul-Sept;77(3):444-450.
- 8.1.8. Fish, RE, Peggy J. Danneman, Marilyn Brown, and Alicia Z. Karas, eds. (2008): Anesthesia and Analgesia in Laboratory Animals, second edition.
- 8.1.9. Green, S., The Laboratory Xenopus sp. CRC Press, 2010. Print edition.
- 8.1.10. Harper, C. and Lawrence, C. The Laboratory Zebrafish. CRC Press, 2011. Print edition.
Institute for Laboratory Animal Research. Guide for the Care and Use of Laboratory Animals, 8th Edition. National Academies Press, 2011.
- 8.1.11. O'Rourke, D and Jenkins, A. Anesthesia and Analgesia in Laboratory Animals (Second Edition), 2008.

8.2. Resources:

8.2.1. Texas A&M University SAPs:

- 8.2.1.1. [15.99.03.M1.03](#) The Responsible Stewardship of Research Data
- 8.2.1.2. [29.01.03.M0.01](#) Security of Electronic Information Resources

8.2.2. IACUC Guidance (TAMU NetID authentication required): <https://rcb.tamu.edu/animals/guidance>

- 8.2.2.1. TAMU-G-002 Guidelines on the use of Anesthesia and Analgesia
- 8.2.2.2. TAMU-G-008 Guidelines for Working with Zebrafish
- 8.2.2.3. TAMU-G-010 Guidelines for the Use of Pharmaceutical and Non-Pharmaceutical Grade Drugs and Compounds
- 8.2.2.4. TAMU-G-035 Guidelines for Performing Surgery in Fish
- 8.2.2.5. TAMU-G-048 Guidelines on Euthanasia of Fish, Amphibians and Reptiles
- 8.2.2.6. TAMU-G-049 Guidelines for Performing Surgery in Amphibians and Reptiles

- 8.3. Please contact [CMP](#) at 979-845-7433 for more information concerning the planning and use of MS222.
- 8.4. Questions regarding safety practices should be directed to [Environmental Health & Safety](#) at: (979) 845-2132, or ehsd@tamu.edu.
- 8.5. Acknowledgements
 - 8.5.1. The document was partially adapted from materials from Florida Atlantic University.

9. HISTORY

Effective Date	Version #	Description
07/18/2019	000	College Station/Galveston: New Document



07/01/2021	001	College Station/Galveston: Content updated to reflect development of new, related guidance.
03/24/2022	002	College Station/Dallas/Galveston: Merging of Dallas animal care and use program with College Station/Galveston
10/20/2022	003	College Station/Dallas/Galveston/Kingsville: Merging of Kingsville animal care and use program with College Station/Dallas/Galveston.
06/01/2024	004	College Station/Dallas/Galveston/Kingsville: Renewal; updated scope and reference sections, addition of records section