



Guidelines for the Preparation and Use of MS222 for Animal Procedures

Reviewed and Approved by FAU IACUC

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Scope

- MS222 or TMS or Tricaine Methanesulfonate is used as an anesthetic and euthanasia agent in fish, amphibians and other cold-blooded animals. This document provides guidance regarding safety for personnel preparing MS222 solutions, the proper usage of the compound, dosages recommended for different applications and proper disposal.
- The products *Finquel* (Argent Chemical Laboratories, Inc., 8702 152 Ave. NE, Redmond, WA 98052, NADA 42-427) and *Tricaine-S* (Western Chemical 1269 Lattimore Road Ferndale, WA 98248, ANADA 200-226) are FDA approved, which the IACUC recommends strongly to use. If other MS222 products are proposed to be utilized the IACUC will request a justification why a non-pharmacological compound has to be used (see also [IACUC Policy 10.4.25](#)). MS222 is the only anesthetic approved for use on fish that could be used as human food and requires a 21-day withdrawal period prior to release or harvest and is only labeled for food fish in the families Ictaluridae, Salmonidae, Esocidae, and Percidae, use with other species should be restricted to the hatchery or laboratory (Bowker et al. 2012).

Safety Precautions

- It is the responsibility of the PI on the corresponding animal use protocol to assure the safe use of MS222 by laboratory staff. The PI is responsible for the appropriate training of research personnel for the safe use of this anesthetic in the species approved on the protocol.
- Personnel using MS222 should be familiar with the MSDS, which should be readily available in the lab. Questions regarding safety practices should be directed to FAU Environmental Health & Safety.
- MS222 is not regulated as a hazardous waste by the US Environmental Protection Agency under the Resource Conservation and Recovery Act. However, users should realize that it is considered an irritant to the eyes, respiratory system and skin and safety precautions need to be employed.
- To avoid inhalation, the fine crystalline powder should be weighed under a fume hood or wearing an N95 mask in a well-ventilated area. To avoid skin and eye contact, goggles, gloves and a lab coat/protective clothing should be worn while measuring the powder. Using a top loading balance with a clear plastic wind/breeze guard can help minimizing the risk of having the light powder blowing around by air currents.



- In typical use, the mode of action is by absorption through the skin of aquatic animals. It can also permeate human skin although at a much lower rate, particularly when dissolved. Personnel should therefore minimize contact with medicated water whenever possible and should be aware and attentive to nausea or headaches after any prolonged exposure of even low concentrated MS222 solution.

Preparation

- Diluents or vehicles must be specified in the animal use protocol.
- MS222 can be administered via immersing the animal in a MS222 solution (i.e. water bath), spraying onto gills with an aerosol pump sprayer in large fish (anesthesia only), or injected directly into the lymph sacs (amphibians) or the coelomic cavity (amphibians and reptiles).
- MS222 can acidify aqueous solutions and therefore solutions may need to be buffered with Sodium Bicarbonate. The investigator needs to consider this possibility and keep the pH of the final solution near the ambient environmental pH for the particular species. Failure to buffer the solution appropriately could result in discomfort/pain to the subjects. For poorly buffered water sources there should generally be a ratio of 1:2 (weight:weight) of MS222 : Sodium Bicarbonate powder.
- MS222 is water soluble and should be prepared in water similar to the natural living conditions of the animal when used as a water bath. Preferably water should be taken from the animal's original holding tank or natural water source. The water should have adequate levels of dissolved oxygen, an appropriate pH, temperature, salinity and hardness.
- Use sterile water or saline and aseptic technique for preparation of MS222 for **injections**. To assure that the product is sterile it must be passed through a syringe filter (0.22 um or finer) at the time of preparation. This can be done in the process of transfer to an injection vial. MS222 for injection has to be prepared fresh each time.
Note that sterile injection vials are available through Comparative Medicine.
- MS222 comes in a powdered form and should be stored at room temperature, in a cool dry place protected from light.
- Use of freshly prepared solutions is recommended, especially if used for surgical procedures to minimize contamination and infection. It has been reported that after 10 days, a 10 percent solution showed a 5% decrease in potency.
- A stock solution can be prepared for use in water bath or spray applications. Commonly used MS222 stock solutions are at 10g/L using aged tap water. Sodium Bicarbonate should be added (10-20g/L).
- Containers must be **labeled** with the agent, concentration, and date of preparation.
- Store preparation in a dark brown bottle or use tinfoil around the bottle. Store in freezer. Discard after one month or earlier if an oily film or discoloration of the stock solution develops.
- Dry MS222 and dry Sodium Bicarbonate should be stored in separate containers since premixed dry ingredients can become hygroscopic and react reducing the effectiveness of the solution.

Use as Anesthetic

- Fast animals for 12-24 hours prior to anesthesia to decrease incidence of regurgitation since MS222 can cause GI response.
- The action of MS222 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.
- Allow animal to reach appropriate level of anesthesia for planned procedure.

- There is a narrower margin of safety in young fish, and soft, warm water. Maintain adequate oxygenation of the animal throughout induction, anesthesia and recovery. Oxygenation of the water can be accomplished by supplying pure oxygen via diffusers or similar devices. Aeration can be accomplished when biomass in the anesthesia container is low by supplying clean air via an air pump and air stone, or similar devices.
- In amphibians, anesthesia induction can be in a water bath. When inducing a terrestrial amphibian in an immersion anesthetic bath, keep the animal's head and nares above the water line to prevent accidental drowning. In some cases, anesthesia with MS222 can be maintained by dripping a dilute solution of this drug over the skin or by covering animals with a paper towel moistened with the anesthetic.
- Higher concentrations of MS222 result in rapid anesthesia with shorter maximum tolerated exposure times, which are recommended for induction.
- Lower concentrations of MS222 result in longer induction times and longer maximum tolerated exposure times, which are recommended for maintenance of anesthesia.
- After procedures are completed place animals in well oxygenated/aerated, un-medicated water or in a container lined with wet towels (terrestrial amphibians).
- Closely monitor fish/amphibians recovering from anesthesia until they are swimming/moving normally and completely regained their righting response.

Recommended Dosages for Anesthesia

Taxonomic Rank	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

- Dosages for MS222 solutions (i.e. water baths) are sometimes provided in different units such ratio, parts per million and percentage weigh/volume.
- Examples for those dosages as per mg/L, ratio (r), parts per million (ppm), percentage weight/volume (%w/v) and percentage % are:
 - 50 mg/L = 1:20,000 (r) = 50 ppm = 0.005 % w/v
 - 100 mg/L = 1:10,000 (r) = 100 ppm = 0.01 % w/v
 - 1 g/L = 1:1,000 (r) = 1000 ppm = 0.1 % w/v

Use for Euthanasia

- MS222 (observe appropriate pH – see preparation section) can be used to euthanize fish, amphibians and reptiles. It is considered an acceptable method per AVMA Guidelines on Euthanasia (2013 edition).
- Fish need to be immersed in concentrated MS222 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).

- Amphibians can be injected with ≥ 250 mg/kg MS222 solution into lymph sacs or intracoelomic. Except for amphibians in life stages with gills, prolonged immersion for up to 1 hour may be required when using a water bath.
- For euthanasia, reptiles can be injected with MS222 into the coelomic cavity. Usually high dosages >500 mg/kg have to be administered that might, however, only lead to anesthesia instead of euthanasia. If MS222 is concentrated above 1% it cannot be buffered with Sodium Bicarbonate anymore since it triggers a strong chemical reaction rendering MS222 ineffective. Therefore, one paper (CJ Conroy et al.) recommends adding a second step injecting a 50% (v/v) MS222 solution (not buffered) after anesthetizing the animal with buffered MS222, which leads to cardiac arrest within 30-60 seconds.
- In any case, death needs to be assured before discarding the animal best by following with a secondary method such as pithing.

Disposal

- Disposal of MS 222 powder and solutions has to be in accord with local authority regulations and is administered through FAU's Environmental Health & Safety department (EH&S).
- Waste solid (MS222 powder, tablets etc) must be sent to EH&S for proper disposal.
- Waste solution (MS222 dissolved in water) has to be diluted with plenty of water (minimum 4:1) and can be drain disposed. If used in the field, it needs to again be diluted (min 4:1) and can be ground disposed but not near waterways.

References

- Bowker, J.D., J.T. Trushenski, M.P. Gaiowski, and D.L. Straus, Editors. 2012. Guide to Using Drugs, Biologics, and Other Chemicals in Aquaculture. American Fisheries Society Fish Culture Section.
- CJ Convoy, 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.
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- AVMA Guidelines for the Euthanasia of Animals: 2013 Edition
- Richard E. Fish, Peggy J. Danneman, Marilyn Brown, and Alicia Z. Karas, eds. (2008): Anesthesia and Analgesia in Laboratory Animals, second edition