IACUC Policy 31: The Use of Tricaine Methanesulfonate (MS-222) in Fishes and Other Aquatic Animals

Policy Purpose: The intent of this policy is to describe the handling, preparation and use of MS-222 at Texas Tech University

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1. Purpose

a. The purpose of this policy is to describe the recommended methods of handling, preparation and use of MS-222. MS-222 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 MS-222 solutions, the proper usage of the compound, dosages recommended for different applications and proper disposal.

2. Usage Policy

a. Formulation: The IACUC strongly recommends a pharmaceutical grade MS222 product. 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 14).

b. Safety Precautions

- It is the responsibility of the PI on the corresponding animal use protocol to assure the safe use of MS-222 by laboratory staff. The PI is responsible for the appropriate training of research personnel for the safe use of this anesthetic/euthanasia agent in the species approved on the protocol. Personnel using MS222 should be familiar with the SDS, which should be readily available in the lab. Questions regarding safety practices should be directed to TTU Environmental Health & Safety.
- Users should realize that MS-222 is considered an irritant to the eyes, respiratory system and skin and safety precautions need to be employed. Accidental ingestion or exposure to the bloodstream may be damaging to the health of the individual by inducing anoxia. Symptoms may not be evident until several hours after exposure.
- To prevent exposure, the powder should be weighed in a fume hood. To avoid skin and eye contact, goggles, gloves and a lab coat/protective clothing should be worn while measuring the powder.
Note: Employees who are required to wear respirators must be in the respiratory protection program.

- 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 MS-222 solution.

c. Preparation

- MS-222 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 required, 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. If used for surgical procedures, preparations must be no more than 48 hours old to ensure potency. All preparations must be discarded within 10 days.
- A stock solution can be prepared for use in water bath or spray applications. Commonly used MS-222 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 MS-222 and dry Sodium Bicarbonate should be stored in separate containers since premixed dry ingredients can become hydroscopic and react reducing the effectiveness of the solution.

d. Use as an Anesthetic

- Solutions for bath immersion are acidic and irritating and must be buffered with sodium bicarbonate to a normal pH (7.4) 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.
- Allow animal to reach appropriate level of anesthesia for planned procedure.
- 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 MS-222 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.
- MS-222 is the preferred anesthetic agent for amphibians and can be administered via immersion in a buffered solution or by intracoelomic injection.
  - 0.1-0.5% (1-5 g/L) for adult Xenopus
  - 0.1-0.2 g/L for tadpoles
e. Use for euthanasia

- MS-222 (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).
- Juvenile and Adult 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 200-300 mg/L is recommended and might be much higher in certain species.
- Amphibians can be injected with ≥250 mg/kg MS-222 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.
  - Xenopus- 5-10 g/L (neutrally buffered solution)
- In any case, death needs to be assured before discarding the animal best by following with a secondary method such as pithing or immersion in liquid nitrogen.

f. Disposal

- Disposal of MS 222 powder and solutions has to be in accord with local authority regulations and is administered through TTU Environmental Health & Safety department (EH&S).
- Waste solid (MS222 powder, tablets etc) must be sent to EH&S for proper disposal.
- Consult EHS for use of MS-222 in the field.

3. References

- Sherril L. Green. 2010. The Laboratory Xenopus.