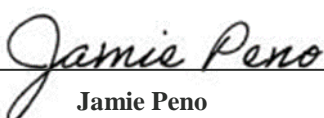
 <div> <div>DIVISION OF RESEARCH & INNOVATION</div> <div>Research Integrity & Compliance</div> </div>		Institutional Animal Care and Use Committee Standard Operating Procedures	
<p align="center">Title: Use of MS-222 in Aquatic Animals</p>			
Effective Date:	December 22, 2020	Document Number:	IACUC-SOP-02-30.00
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REVISION HISTORY			
Date	Section	Author	

PURPOSE

Tricaine Methanesulfonate (MS-222) is used as an anesthetic and euthanasia agent in fish, amphibians, and other cold-blooded animals. This document provides guidance regarding the safety of personnel preparing MS-222 solutions, the proper usage of the compound, dosages recommended for different applications, and proper disposal.

SCOPE

It is the responsibility of the Principal Investigator to include the use of MS-222 in the IACUC protocol to be approved before it can be used.

It is the responsibility of the Principal Investigator to follow guidelines outlined in this procedure and ensure staff using these methods are approved to do so in the IACUC approved animal use protocol and training has been performed and appropriately documented.

DEFINITIONS AND ABBREVIATIONS

UNT- University of North Texas, Denton

IACUC- Institutional Animal Care and Use Committee

NPGS(s)- Non-Pharmaceutical Grade Substance(s)

SOP/SOP's- Standard Operating Procedure(s)

AUP- Animal Use Protocol

PI- Principal Investigator

AV- Attending Veterinarian

MS-222- Tricaine methanesulfonate

PROCEDURES

- I. Formulation of MS-222**
 - A.** The IACUC strongly recommends using a pharmaceutical-grade MS-222 product; however, if other MS-222 products are to be utilized, justification for the use of a non-pharmaceutical grade compound must be provided in the IACUC protocol.
- II. Safety Precautions**
 - A.** Personnel using MS-222 should be familiar with the SDS, which should be readily available in the laboratory. Questions regarding safety practices should be directed to UNT Risk Management.
 - 1. MS-222 is considered an irritant to the eyes, respiratory system, and skin. Safety precautions should be employed when used.
 - 2. 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.
 - 3. To prevent exposure, the powder should be weighed in a fume hood. To avoid skin and eye contact, goggles, gloves and lab coat/ protective clothing should be worn while measuring the powder. Employees who are required to wear respirators must be cleared through occupational health, to ensure proper mask fitting and testing.
 - 4. 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 be aware and attentive to nausea or headaches after any prolonged exposure of even low concentrated MS-222 solution.
- III. Preparation**
 - A.** MS-222 comes in a powdered form and should be stored at room temperature, in a cool dry place protected from light.
 - B.** 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.
 - C.** A stock solution can be prepared for use in a 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).
 - 1. Containers must be labeled with the agent, concentration, and date of preparation.
 - 2. Preparation should be stored in a dark brown bottle or use tinfoil around the bottle and should be stored in the freezer.
 - 3. Discard after one month or earlier if an oily film or discoloration of the stock solution develops.
 - D.** Dry MS-222 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.
- IV. Use as an Anesthetic**
 - A.** Solutions for bath immersion are acidic and irritating, and therefore, must be buffered with sodium bicarbonate to a normal pH (7.4) before use.
 - B.** 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.
 - C.** Allow animal to reach appropriate levels of anesthesia for the planned procedure.
 - D.** 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.

- E.** MS-222 is the preferred anesthetic agent for amphibians and can be administered via immersion in a buffered solution or by intracoelomic injection.
 - 1. 0.1-0.5% (1-5 g/L) for adult *Xenopus*
 - 2. 0.1-0.2 g/L for tadpoles
- F.** Tricaine is the only anesthetic approved in the U.S. FDA for fish intended for food and is the most commonly used agent for the anesthesia of fish.
 - 1. 25-100 mg/L (Zebrafish 100 mg/L)
- G.** After procedures are completed, place animals in well-oxygenated/ aerated, un-medicated water. Alternatively, animals may be placed in a container lined with wet towels, especially for terrestrial amphibians.
- H.** Closely monitor fish/amphibians recovering from anesthesia until they are swimming/ moving normally, and completely regained their righting response.

V. Use for euthanasia

- A.** MS-222 (observe appropriate pH-see preparation section) can be used to euthanize fish, amphibians, and reptiles. It is considered an acceptable method per the AVMA Guidelines on Euthanasia.
 - 1. 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.
 - 2. 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 one hour may be required when using a water bath.
 - a) *Xenopus*: 5-10g/L (neutrally buffered solution)
- B.** 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.

VI. Disposal

- A.** Disposal of MS-222 powder and solutions has to be in accord with local authority regulations, and in consultation with UNT Risk Management.

REFERENCES

- 1. The Guide for the Care and Use of Laboratory Animals.
- 2. AVMA Guidelines for Euthanasia

APPENDICES

IACUC Standard Operating Procedures