University of Maryland Center for Environmental Science (UMCES)
Institutional Animal Care & Use Committee (IACUC)
Guideline & Standard Operating Procedure
for
Tricaine methane sulfonate (MS-222, Finquel, TMS)
Version: October 2, 2017
Adopted: November 10, 2017

The intent of this Institutional Animal Care and Use Procedure (IACUP) is to describe common anesthesia and euthanasia procedures for fish and amphibians. This IACUP has been approved by the IACUC. Investigators may refer to this in their IACUC submissions. Any deviation from the procedures below must be detailed in the IACUC submission and approved by the IACUC prior to its implementation.

1. Introduction

a. Tricaine methane sulfonate (MS-222, TMS or Finquel) is used at UMCES as an anesthetic and euthanasia agent in fish and amphibian species. As such, it poses two issues of concern to the IACUC: 1) its safe preparation and use by animal handlers and 2) not being of pharmaceutical grade.
b. This document provides guidelines for fish and amphibian anesthesia and euthanasia procedures, safety, and post-anesthetic care and monitoring for the use of tricaine methane sulfonate.
c. MS-222 is an FDA approved fish anesthetic (FDA ANADA 200-226) and has a mandated withdrawal time of 21 days after its use in food fish.
d. MS-222 is not regarded as a toxic chemical by OSHA classification. Nevertheless, precautions should be taken when handling it, because as a fine powder it is a respiratory irritant. Also, some literature suggests that MS-222 is a potential mutagen/carcinogen. MS-222 should also be used with caution because it can cause retinol toxicity for people working with it.[1]

2. Materials

a. Anesthetic agent [e.g. reagent or pharmaceutical grade tricaine methane sulfonate (MS-222) depending on availability], sodium bicarbonate (if using MS-222).
b. Chemical Gloves (e.g. Nitrile): When handling animals, wear non-powdered gloves pre-moistened with distilled or de-chlorinated water)
c. Transport, anesthetic and recovery tanks
d. Oxygenation equipment (e.g. air pump, tubing, and air stone)

3. Procedures

a. General considerations
    i. If using a new anesthetic protocol or species, anesthetize a small cohort of animals and follow them through full recovery to ensure drug dosages and techniques are safe, and provide sufficient anesthetic depth for the

intended procedures.

ii. Do not disturb the mucus layer of fish or amphibians. Wear non-powdered, pre-moistened gloves when handling animals. Do not apply detergents or solvents to the skin, and limit contact with abrasive materials (e.g. dry paper towels)

iii. Buffer anesthetic solutions by adding food grade baking soda (sodium bicarbonate) in a ratio of 1:1 to tricaine methane sulfonate and water.

b. Fish Anesthesia –

vi Use water taken from original fish holding tank for transport, anesthetic and recovery chambers. If using another water source, closely duplicate the water quality parameters (i.e., chlorine, temperature, pH, and salinity) of the original holding tank. Buffer water to a pH of 7.0 – 8.0 using sodium bicarbonate after addition of MS-222 and before addition of fish.

vii Maintain adequate oxygenation of holding tanks throughout induction, anesthesia, and recovery.

a) Supply oxygen via air pump and air stone, or similar device

viii Maintain water temperature at the species' normal optimum during both anesthesia and recovery.

ix Anesthesia is achieved by immersion in an anesthetic solution to achieve the desired stage of sedation/ anesthesia.

Stage 1: Deep sedation	Stage 2:Deep narcosis	Stage 3: Surgical anesthesia
• Cessation of voluntary swimming • Decreased response to stimuli.	• Decreased muscle tone • Equilibrium loss • Appropriate level for fin and gill biopsies.	• Slow respiration and heart rate • Total loss of response to stimuli • Firmly squeeze at the base of the tail to determine response to stimuli.

x Dosage: Allow animal to reach appropriate level of anesthesia for planned procedures. Suggested dosages are-

a) *Surgical anesthesia*: 100 mg/liter buffered tricaine methane sulfonate. Anesthetic doses for fish typically start at 100 mg/liter tricaine methane sulfonate but may need to be increased dependent on the species. Once immersed in a well-aeriated, buffered solution the surgical plane of anesthesia is usually reached in approximately 2-4 minutes. If the fish is not anesthetized, slowly increase the concentration of the anesthetic solution. After the fish has reached stage 3 above, it can then be removed from the solution; anesthesia is maintained by dripping solution onto the gills or returning the animal to the anesthetic tank.

b) *Tranquilization and transport*: 20-50 mg/liter buffered tricaine methane sulfonate buffered

xi While performing procedures, keep the fish's skin moist and the gills submerged or regularly flushed with well oxygenated water that contains anesthetic, as needed.
   a) Evaluate respiratory rate and gill color throughout anesthesia: Observe movement of the operculum (rigid flap that covers the gills) as it opens and closes to assess rate.
   b) Observe gill color; should be dark pink to light red.
   c) If respirations become extremely slow or stop, place the fish in anesthetic-free recovery water or flush the gills with anesthetic-free water until respirations resume

xii Recover the fish from anesthesia by placing it into a recovery tank containing well aeriated water, or by flushing the gills with water.

c. <u>Amphibian Anesthesia –</u>

vi. Use water taken from the original holding tank for transport, anesthetic and recovery chambers. If using another water source, closely duplicate the water quality parameters (i.e., chlorine, temperature, pH and ammonia) of the original holding tank. . Buffer water to a pH of 7.0 – 7.1 using sodium bicarbonate after addition of MS-222 and before addition of amphibian.

vii. Maintain temperature at the species' normal optimum during both anesthesia and recovery.

viii. Anesthetic induction may produce an excitement phase. Induce anesthesia in a container that will preclude injury from the animal jumping or falling out.

ix. Anesthesia is achieved by immersion in an anesthetic solution.

x. Stages of anesthesia in amphibians:

Induction	Light anesthesia	Surgical anesthesia
• Decreased gular movement • Diminished withdrawal reflex	• Loss of righting reflex • Absence of abdominal respirations	• No withdrawal reflex (toe pinch) • Gular movements cease

Note: Pulmonary respiration may cease under a surgical plane of anesthesia. Cutaneous respiration is sufficient to prevent clinical hypoxia during anesthesia. Heart rate can be monitored via direct observation, or using a Doppler flow detector placed over the heart.

vi. Dosage: Allow animal to reach appropriate level of anesthesia for planned procedures. Suggested dosages are-

a). Adult frogs: Surgical anesthesia can be induced with an initial dose of 0.5 to 2 g/liter over a 10-30 minute period. Maintenance: reduce the dosage as low as possible, some species may remain anesthetized with as low as 100-200 mg/L.

b). Tadpoles: 0.2 to 0.5 g/liter

vii. Allow animal to reach appropriate level of anesthesia for planned procedures

viii. Remove the animal from the anesthetic bath after appropriate level of anesthesia is reached. Keep the amphibian's skin moist throughout procedures.

ix. If supplemental anesthesia is needed, anesthetic solution can be dripped onto the animal's skin

d. Post anesthetic Care
   i. General consideration
      - Closely monitor fish/amphibians recovering from anesthesia until they are swimming/moving normally and have completely regained their righting response.
   ii. Fish
      - Place the fish in well oxygenated, un-medicated water in a holding tank. (a) Moving the fish back and forth in the water (b) Or, opening and closing the mouth several times
      - Maintain water temperatures at the species' normal optimum throughout recovery
   iii. Amphibians
      - After procedures are completed, rinse the animal with fresh water.
      - Recovery Chamber for Aquatic Species – place animal in well oxygenated, un-medicated water in a holding tank.
      - Recovery Chamber for Terrestrial Species- place animal in either.
        - Un-medicated water in a holding tank (keep the animal's head and nares above the water line to prevent accidental drowning),
        - or in a container lined with wet towels
      - Do not raise the amphibian's body temperature above that of the species' normal optimum in an attempt to speed recovery.
        - Increased body temperature will increase metabolism and oxygen requirements.
        - Cutaneous respiration may not be sufficient to maintain adequate oxygenation in this situation.

EUTHANASIA

Fish

The fish is overdosed by immersion in 300-500 mg/liter tricaine methane sulfonate buffered to pH 7.0-7.5 for no less than 10 minutes following cessation of operculum movements. A secondary method such as decapitation followed by pithing must be used as recent studies have shown some animals to recover from the doses listed above.

Amphibians

The amphibian is overdosed by immersion in 5-10 gms/liter tricaine methane sulfonate buffered to pH 7.0-7.5 for up to an hour. This must be followed by an adjunctive method of euthanasia; either pithing or decapitation. Decapitation can be performed with heavy shears or a guillotine, and must be followed by pithing or another method of destroying brain tissue. The pithing site in frogs is the foramen magnum, which can be identified by a slight midline skin depression posterior to the skull, midline between the eyes, with the neck flexed.

References:

1. National Institute of Health, “Retinal toxicity associated with occupational exposure to the fish anesthetic MS-222”: http://www.ncbi.nlm.nih.gov/pubmed/9402834