

Algoma University Animal Care Committee	
AU #0007 Anesthesia or Euthanasia of Fish using MS-222 (adapted from Carleton University's SOP VM 003)	
Issue date: June 25, 2013	Last revised: October 2024

1. Purpose

In studies on fish, there is the occasional need to use a general anesthetic to immobilize them and to reduce the level of stress associated with certain relatively non-invasive handling procedures such as body length and weight measurements, digital photography of the body (e.g. for quantification of body colouration) and individual tagging, for example. Moreover, anesthetic overdose can also be used as an effective and humane method of euthanizing fish when necessary.

Use of tricaine methanesulfonate (TMS, also known as MS-222) as a general fish anesthetic, either in very dilute concentration for tagging and other relatively non-invasive (non-surgical) procedures or in overdose concentration for euthanasia.

As recommended by the CCAC's "Guidelines on the care and use of fish in research, teaching and testing (2005)", and its supplementary article on "Anesthetics" by Ackerman, Morgan & Iwama, anesthetics should be used in studies of fish that entail "extensive handling or manipulation with a reasonable expectation of trauma and physiological insult to the fish". These documents state that the use of anesthetics is primarily for holding fishes immobile while being handled and also to lower the level of stress associated with a number of procedures.

The choice of anesthetic depends on several factors. However, TMS (MS-222) is currently only one of two chemical anesthetics registered for veterinary use with fishes in Canada. According to Ackerman et al., MS-222 is the most widely used fish anesthetic, and it is extremely effective for rapid induction of deep anesthesia. A lethal dose of buffered MS-222 is also a preferred method of euthanasia for fishes according to CCAC guidelines (2005).

NOTE: Syndel is the only approved supplier of MS-222 in Canada and requires a prescription from a veterinarian to order the chemical. This can be provided by the veterinarian who is part of the animal care committee.

NOTE: Consult the MSDS for MS-222 and sodium bicarbonate prior to handling of these chemicals and ensure you use the appropriate PPE.

NOTE: MS-222 baths can quickly break down in sunlight and should be made fresh daily. DO NOT store made-up MS-222 solutions for later use.

2. Policy

CCAC guidelines and regulations

3. Responsibility

It is the responsibility of the Principal Investigator to ensure that this SOP is followed and that those handling MS222 are appropriately trained to do so and have read the accompanying SDS.

4. Training Required

WHMIS

Animal Research Ethics

Handling/training in the relevant species

Bio-methodology training in the relevant technique and species

Registered Veterinary Technology Certification or equivalent

5. Materials Required

Gloves

Safety eye glasses/goggles

Dust mask

Lab coat

Dipnet

Bottle of TMS (MS-222). Supplier: Syndel International Inc., Vancouver, BC

Top-loading weighing balance

Weighing "boat"

Spatula

Glass stirring rod

Container of aged tap water for the preparation of the MS-222 solution

Anesthesia container containing an appropriate volume of prepared MS-222 solution to euthanize the fish (approximating the ambient temperature of the source of water from which the fish originate)

Recovery aquarium containing aerated aged water at same temperature as the MS-222 solution (and stock holding aquaria)

Refrigerator (for storage of prepared stock solution of MS-222)

Eyewash station in case of accidental exposure to eyes

Soap and water in case of accidental exposure to skin

Broom and shovel if required for safe clean up

6. Procedures

NOTE: The concentration of MS-222 used for anesthesia can be different for different species of fish and can depend upon water chemistry (such as alkalinity and hardness). Therefore it is advisable to start with a low concentration of MS-222 and increase if needed. The animal should be anesthetized in 5 minutes or less; if this is not the case a higher concentration of MS-222 is required.

A. For non-lethal light anesthesia

1. Fill a measured amount of dechlorinated or aged tap water into the container for the anesthetic bath 2)
2. Add 100-150 mg of MS-222 per L of water to the container (as stated above the concentration of MS-222 can be dependent on fish species and water chemistry)
3. Add twice as much sodium bicarbonate to the container (e.g., if you add 120 mg of MS-222 to the container add 240 mg of sodium bicarbonate)
4. Stir the anesthetic bath with a stirring rod or net to ensure both chemicals have fully dissolved

5. Set up a recovery bath filled with dechlorinated or aged tap water
6. Using a net, transfer the fish to be anesthetized to the anesthetic bath
7. Constantly monitor the fish.
 - a. For light anesthesia the animal will start to lose balance in the water column, at this point the animal can be removed and measured or a procedure performed in accordance with the AUP
 - b. If deeper anesthesia is required (such as it is with performing electro-olfactograms or more invasive surgeries) wait until the animal is on its side and opercular movement has stopped (NOTE: when opercular movement stops the animal is no longer breathing and must quickly have water moving over its gills to survive)
8. Once the procedure is complete, return the animal to the recovery bath
9. Monitor the animal until it has recovered. If the opercula are not moving, put on gloves, grasp the animal by the tail and move it back and forth to push water through its gills
10. Return animal to holding tank after it is able to stay upright in the water column, its opercula are moving, and it is able to swim

Concentrations for light anaesthesia by species:

Species	Concentration
Fathead minnows (<i>Pimephales promelas</i>)	100-150 mg/L

B. For euthanasia

This procedure is to euthanize fish, either as part of an experiment or because a humane endpoint has been reached. NOTE: The concentration of MS-222 used for euthanasia can be different for different species of fish and water hardness. That being said, a concentration of 250 mg/L MS-222 is usually sufficient to quickly euthanize most fish.

1. Fill a measured amount of dechlorinated or aged tap water into the container for euthanizing the fish
2. Add 250 mg of MS-222 per L of water (NOTE: use a higher concentration if needed)
3. Add 500 mg of sodium bicarbonate to the container per L of water. The solution should now be of pH 7.0-7.5
4. Stir the euthanasia bath with a stirring rod or net
5. Using a net, transfer the fish to be euthanized to the bath
6. Constantly monitor the fish, remove when the the animal is on its side and all movement has stopped
7. Perform a secondary form of euthanasia such as a blow to the head or decapitation 8) Transfer the carcass to a freezer for storage prior to disposal as per the AUP

Concentrations for euthanasia by species as specified by Fisheries and Oceans Canada (GWACC-107 – Fish Euthanasia)

Species	Concentration
Eastern Sand Darter (<i>Ammocrypta pellucida</i>)	250 mg/L
Johnny Darter (<i>Etheostoma nigrum</i>)	250 mg/L
Fathead minnows (<i>Pimephales promelas</i>)	250 mg/L

7. References (if applicable)

- (i) CCAC document “Guidelines on the care and use of fish in research, teaching and testing (2005)”, and its accompanying supplementary article on “Anesthetics” by Ackerman, Morgan & Iwama.
- (ii) Syndel Laboratory Ltd. “Safety and First Aid Information Sheet for TMS”

REVISION HISTORY		
Revision #	Revision Date	Summary of Changes
1	May 1, 2019	
2	April 23, 2023	Suggestion to make this species specific by veterinarian
3	May 2, 2024	
4	July 15, 2024	Reformatted to new SOP template
5	October 10, 2024	Revised committee approved via email concensus
6	October 30, 2024	Revision accepted at Fall 2025 meeting - species specific doses added