

Standard Operating Procedures

AU #0007 Anaesthesia or Euthanasia of Fish using MS-222 (adapted from Carleton University's SOP VM 003)

Purpose

In studies on fish, there is the occasional need to use a general anaesthetic 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, anaesthetic 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 anaesthetic, 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, anaesthetics 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 anaesthetics is primarily for holding fishes immobile while being handled and also to lower level of stress associated with a number of procedures.

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

Responsibilities

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.

Materials

Latex 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

Anaesthesia 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

Supporting Documentation:

(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”

PROCEDURES

A. For non-lethal light anaesthesia

1. Wear latex gloves and work under a fume hood at all times when handling chemicals
2. Also wear a disposable surgical mask, eye goggles and lab coat when handling the MS-222 powder in air and when preparing the MS-222 solution
3. Prepare a stock buffered MS-222 solution as follows:
 - a. weigh 0.08 g of MS-222 powder in a weighing “boat”
 - b. weigh 0.16 g of sodium bicarbonate in a weighing “boat”
 - c. add the weighed MS-222 and sodium bicarbonate to 1 litre of aged tap water, and mix thoroughly
 - d. pour this buffered MS-222 solution into a labelled plastic bottle
 - e. refrigerate this bottled stock MS-222 solution until needed (but within 1 month of preparation).
4. To begin anaesthetizing fish, pour an appropriate volume of the prepared stock MS-222 solution into the anaesthesia container
5. Return the stock MS-222 solution to the refrigerator
6. Using a water bath, bring the temperature of the MS-222 solution in the anaesthesia container to the ambient temperature of the source of water from which the fish originate
7. Dipnet one fish from the source water and place into the anaesthesia container
8. Continuously observe the fish and gently remove it by hand from the anaesthesia container when it has lost its equilibrium and is quiescent (this normally takes less than 3 minutes)
9. Promptly carry out the relevant procedure (e.g. measuring, tagging) on the anaesthetised fish. Keep the fish moist during the procedure using a wet paper towel

10. Once procedure is completed, place the anaesthetized fish into the recovery aquarium (containing well-aerated,(Stress-Coat) conditioned and aged tap water at same temperature as anaesthesia solution)
11. Monitor the fish's behaviour during recovery; the fish should return to normal swimming activity within 10 minutes.
12. Repeat steps #7-11 for additional fish to be anaesthetized
13. Once the anaesthetic procedure has been completed on all fish, store the used anaesthetic solution in the on-site waste storage facility until RPR arrives to collect it for safe disposal
14. Discard the latex gloves
15. Following full recovery, the fish can be transferred back to their home holding aquarium or experimental aquaria, as required by the experimental protocol.

B. For euthanasia

1. Wear latex gloves and work in a well-ventilated area at all times
2. Also wear a dust mask, eye goggles and lab coat when handling the MS-222 powder in air and when preparing the MS-222 solution
3. Prepare a stock of buffered MS-222 (lethal dose) solution as follows:
 - a. weigh 1 g of MS-222 powder in a weighing "boat"
 - b. weigh 2 g of sodium bicarbonate in a weighing "boat"
 - c. add the weighed MS-222 and sodium bicarbonate to 1 litre of aged tap water, and mix thoroughly
 - d. pour this buffered MS-222 solution into a labelled plastic bottle
 - e. refrigerate this bottled stock MS-222 solution until needed (but within 1 month of preparation).
4. To begin euthanizing fish, pour an appropriate volume of the prepared stock MS-222 lethal-dose solution into an euthanasia container
5. Return the stock MS-222 lethal-dose solution to the refrigerator
6. Using a water bath, bring the temperature of the MS-222 solution in the euthanasia container to the ambient temperature of the source of water from which the fish originate
7. Dipnet one or more fish to be euthanized from the source water and place into the euthanasia container
8. The fish should be dead within 10 minutes. Verify by ensuring that all opercular and bodily movements have ceased completely
9. Remove dead fish from the container and place in a plastic bag
10. Place the plastic bag in the lab freezer for later disposal following accepted University procedures
11. Discard the used MS-222 euthanasia solution.
12. Discard the latex gloves

C. For Disposal

1. Collect using on the site waste storage facilities until RPR arrives to collect it for safe disposal

Note: Do not discard MS-222 directly into surface water, stormwater conveyances or catch basins. Discharge into the environment must be avoided.

D. For Accidental Exposure

1. Ensure adequate ventilation and avoid inhaling any dust formed.
2. Evacuate all personnel to safe areas
3. In case of skin contact, wash with soap and water
4. In case of eye contact, utilize eye wash station and rinse eye thoroughly for at least 15 minutes
5. If swallowed, rinse mouth with water and consult a physician

E. For Accidental Release

1. Wearing safety equipment, prevent further release if it is possible to safely do so
2. Avoid discharging the chemical into the environment
3. Collect spilled materials using a broom or shovel
4. Avoid inhaling any dust formed
5. Gather all spilled materials in the on-site waste disposal facilities until RPR arrives to collect them for safe disposal

ADDITIONAL COMMENTS

For studies that involve mark-recapture of wild fishes (with ACC-approved protocols), fish that have been tagged under light MS-222 anaesthesia (following the procedures outlined above) will be held in aquaria in the laboratory at temperatures of about 20C to recover for at least 5 days before being released live into the field (either lake or stream/river) at their original site of capture. At this holding temperature and holding period duration, this procedure meets the Fisheries & Oceans Canada's (DFO) recommended withdrawal period for MS-222.

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