Fish Anaesthesia

This SOP (Standard Operating Procedure) describes the procedure for anesthetizing fish. It is approved by the NUS Institutional Animal Care and Use Committee (IACUC). Deviations from this SOP must be approved by the IACUC prior to implementation, or upon recommendations by a CM Veterinarian.

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1. INTRODUCTION

This document provides guidelines for fish anesthesia procedures, post-anesthetic care and monitoring.

2. MATERIALS

a. Anesthetic agent - Tricaine Methane Sulfonate (MS-222) or 2-phenoxyethanol (when possible, please use drugs of pharmaceutical grade)
   i. MS-222
      1. It is the methanesulfonate of meta-amino benzoic acid ethyester, or simply ethyl m-amino benzoate.
      2. It is intended for the temporary immobilization of fish, amphibians and other aquatic, cold-blooded animals, and is the only anesthetic approved by the Food and Drug Administration (FDA) for use with food fish in the United States.
      3. It is a fine, white crystalline powder and forms a clear, colourless, acidic solution in water.
      4. The colour of MS-222 solution may change rapidly to yellow or brown when exposed to sunlight but this does not affect its
5. activity in any significant way, unless the solution has been kept for more than 3 days. For best results, use freshly prepared solution.

ii. 2-phenoxethanol
   1. It is ethylene glycol monophenyl ether.
   2. It is a colourless, oily liquid with a pleasant smell.
   3. It is used as a fixative for perfumes and acts as a bactericidal agent in conjunction with quarternary ammonium compound. It is also used as an insect repellant.
   4. It is used as an anesthetic in aquaculture of some fish.

b. Buffering agent - Sodium Bicarbonate powder
c. Gloves (when handling animals, wear non-powdered gloves pre-moistened with distilled or de-chlorinated water)
d. Species-appropriate transport, anesthetic and recovery tanks
e. Oxygenation equipment (e.g. air pump, tubing and air stone) to ensure adequate aeration of water, especially with the use of MS-222 which is a hypoxic agent. These oxygenated equipment are available in individual aquarium at DBS as well as Pediatrics Fish Facility.
f. pH paper for testing MS-222 solution

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 the fish. 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. If you need help on anesthesia for your IACUC protocol, contact a CM Veterinarian for assistance.

b. Fish anesthesia
   i. Use water taken from original fish holding tank for transport, anesthetic and recovery tanks. If using another water source, closely duplicate the water
quality parameters (i.e. chlorine, temperature, pH and ammonia) of the original holding tank.

ii. Maintain adequate oxygenation of holding tanks (i.e. supply oxygen via air pump and air stone or similar devices) throughout induction, anesthesia and recovery.

iii. Maintain water temperature at the species’ normal temperature during both anesthesia and recovery. The normal and optimum temperature is species-specific but in general, the accepted optimum temperature for zebrafish and medaka is approximately 28°C.

iv. Anesthesia is achieved by immersion in the MS-222 solution. Always buffer solution with an equal weight of sodium bicarbonate to maintain neutral pH.
   1. For inducing anesthesia, measure 75-125mg of MS-222 powder on an appropriate weighing scale.
   2. Add the powder into 1L of water, ensuring that the water is adequately aerated with one of the oxygenation equipment stated above.
   3. Measure an equal amount of sodium bicarbonate (e.g. if 75mg of MS-222 were measured and added into the water, 75mg of sodium bicarbonate powder will be needed as a buffering agent).
   4. Add the sodium bicarbonate in the MS-222 solution.
   5. Use pH paper to assess pH of the anesthetic solution. The ideal pH of the anesthetic solution should be about 7.2-7.5.

v. Dose of MS-222 for induction is 75-125mg/L of buffered aqueous solution and dose for maintenance is 50-75mg/L of buffered aqueous solution.

vi. Dose of 2-phenoxyethanol is 0.1-0.5ml/L. (Note: This drug has a narrow safety margin as an anesthetic agent and should be used with caution. It is recommended that the drug be used at the lower dose, for less invasive procedures like gill or fin biopsies.)

vii. Allow the fish to reach appropriate level anesthesia appropriate for planned procedure – See Appendix A ‘Stages of Anesthesia in Fish’.

viii. While performing the procedures, keep the fish’s skin moist and the gills submerged or regularly flushed with well-oxygenated water.

ix. Evaluate respiratory rate and gill colour throughout anesthesia.

x. The respiratory rate can be assessed by observing the operculum (rigid flap that covers the gills) as it opens and close. The gill colour should remain dark pink to light red.

xi. If respiration stops or becomes very slow, place the fish in anesthetic-free recovery water until respiration resumes.
c.  Post-anesthetic care
   a.  General Considerations
      i.  Monitor the fish recovering from anesthesia closely until they are
          swimming normally and have completely regained their righting
          response.
   b.  Fish care
      i.  Place the fish in well-oxygenated, unmedicated water in a recovery tank.
      ii. To speed up recovery, create a flow of oxygenated water over the gills by
          either moving the fish back and forth in the water, OR by opening and
          closing its mouth several times.
      iii. Maintain water temperature at the species’ normal temperature
          throughout recovery. The normal and optimum temperature is species-
          specific but in general, the accepted optimum temperature for zebrafish
          and medaka is approximately 28°C.

4.  PERSONNEL SAFETY

When working with animals, wear appropriate PPE, observe proper hygiene and
be aware of allergy, zoonosis and injury risks. Refer to the OSHE webpage
(http://www.nus.edu.sg/osh/) for more information.

a.  Medical Emergencies: Please refer to the Occupational Health Clinical Services
    at University Health Centre (UHC) or NUH (National University Hospital).

b.  2-phenoxyethanol safe practices:
    i.  Handle chemical inside fume hood where possible. Otherwise, respirators
        should be worn if handled outside a fume hood.
    ii. Wear protective clothing (e.g. lab coat) and gloves to prevent skin
        exposure.
    iii. Wear appropriate protective eyeglasses or chemical safety goggles.
    iv.  Residue poses a fire risk and empty containers should placed in a fume
        hood to evaporate the residue.
    v.   Wastes must be disposed as chemical waste.

c.  MS-222 safe practices:
    i.  Wear protective clothing, gloves and goggles when handling MS-222
        powder and wear gloves to handle animals exposed to MS-222.
    ii. Whenever possible, work inside a fume hood to prepare a concentrated
        stock solution by mixing an appropriate amount of MS-222 powder in a
        small volume of water. Wear gloves and use a utensil to stir until all
powder is dissolved. Dilute the stock solution further as required. Avoid inhaling MS-222 or getting it into the eyes.

iii. Its use should be limited to hatcheries or laboratories. Do not use within 21 days of harvesting fish for food.

iv. MS-222 wastes should be disposed as chemical waste unless it is processed into a non-hazardous substance before disposal.

5. ANIMAL RELATED CONTINGENCIES

a. Post contact information for emergency assistance in a conspicuous location within the animal facility.

b. Emergency veterinary care is available at all times including after working hours, weekends and public holidays through the emergency vet phone 90013073.

6. REFERENCES


7. APPENDIX

Stages of Anesthesia in Fish

<table>
<thead>
<tr>
<th>Stage 1: Deep sedation</th>
<th>Stage 2: Deep narcosis</th>
<th>Stage 3: Surgical Anesthesia</th>
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<tbody>
<tr>
<td>• Cessation of voluntary swimming.</td>
<td>• Decreased muscle tone</td>
<td>• Slow respiration and heart rate</td>
</tr>
<tr>
<td>• Decreased response to stimuli.</td>
<td>• Loss of equilibrium</td>
<td>• Total loss of response to stimuli (firmly squeeze base of tail to determine response to stimuli).</td>
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<tr>
<td></td>
<td>• This stage of anesthesia is appropriate for fin and gill biopsies</td>
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