FISH AND AMPHIBIAN ANESTHESIA

1. PURPOSE

1.1. The intent of this Animal Care and Use Procedure (ACUP) is to describe common anesthesia procedures for fish and amphibians. This ACUP is approved by the Cornell IACUC. Any deviation must be approved by the IACUC prior to its implementation.

2. SCOPE

2.1. This ACUP is intended for use by investigators and staff who have approval to anesthetize fish and amphibians in their study protocol.

3. INTRODUCTION

3.1. This document provides guidelines for fish and amphibian anesthesia, procedures, and post-anesthetic care and monitoring. Contact CARE at care@cornell.edu for more information or for other assistance.

4. MATERIALS AND EQUIPMENT

4.1. Anesthetic agent (e.g., pharmaceutical grade tricaine methanesulfonate [MS-222]), sodium bicarbonate (if using MS-222).
4.2. Gloves (powder-free, pre-moistened).
4.3. Transport, anesthetic, and recovery tanks.
4.4. Oxygenation equipment (e.g., air pump, tubing, and air stone).

5. PROCEDURE

5.1. General Considerations

5.1.1. If using a new anesthetic protocol or species, anesthetize a small cohort of animals. Follow them through to recovery to ensure drug dosages and techniques are safe and provide sufficient anesthetic depth for the intended procedures.
5.1.2. Do not disturb the mucus layer of fish or amphibians. When handling animals, wear powder-free gloves pre-moistened with distilled or dechlorinated water. Do not apply detergents or solvents to the animal’s skin, and limit contact with abrasive materials (e.g. dry paper towels).

5.1.3. Ensure that all water used for fish and amphibians is well-oxygenated, chlorine-free (dechlorinated or distilled), and within the normal temperature range for the species.

5.1.4. For help designing an anesthetic protocol contact CARE at care@cornell.edu.

5.2. Fish Anesthesia

5.2.1. Fast fish for 12–24 hours prior to anesthesia.

5.2.2. When possible, 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 ammonia) of the original holding tank.

5.2.3. Anesthesia is achieved by immersing the animal in an anesthetic solution (see Appendix for a partial list of agents and doses).

5.2.4. 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>
</tr>
</thead>
<tbody>
<tr>
<td>• Cessation of voluntary swimming</td>
<td>• Decreased muscle tone</td>
<td>• Decreased respiration and heart rate</td>
</tr>
<tr>
<td>• Decreased response to stimuli</td>
<td>• Equilibrium loss</td>
<td>• Total loss of response to stimuli</td>
</tr>
<tr>
<td></td>
<td>• Appropriate level for fin and gill biopsies.</td>
<td>• Firmly squeeze at the base of the tail to determine response to stimuli.</td>
</tr>
</tbody>
</table>

5.2.5. Allow animal to reach appropriate level of anesthesia prior to beginning procedures.

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

5.2.7. Evaluate respiratory rate and gill color throughout anesthesia:

5.2.7.1. Observe movement of the operculum (rigid flap that covers the gills) as it opens and closes to assess rate.

5.2.7.2. Observe gill color; should be dark pink to light red.

5.2.7.3. If respirations become extremely slow or stop, place the fish in anesthetic-free recovery water until respirations resume. If the fish must be re-anesthetized after recovery, proceed with a decreased concentration of MS-22 and monitor respiration closely.

5.3. Amphibian Anesthesia

5.3.1. Fast for 12–24 hours prior to anesthesia.
5.3.2. When possible, 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.

5.3.3. Anesthetic induction may produce an excitement phase and must be performed in a container that will prevent the animal from jumping or falling out.

5.3.4. Anesthesia is achieved by immersing the animal in an anesthetic solution (see Appendix for a partial list of agents and doses).

5.3.4.1. If alternative methods of anesthesia are desired, contact CARE at care@cornell.edu for assistance.

**NOTE:** 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).

5.3.5. Stages of anesthesia in amphibians:

<table>
<thead>
<tr>
<th>Induction</th>
<th>Light Anesthesia</th>
<th>Surgical Anesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Decreased gular movements</td>
<td>• Loss of righting reflex</td>
<td>• Loss of withdrawal reflex (toe pinch)</td>
</tr>
<tr>
<td>• Diminished withdrawal reflex</td>
<td>• Absence of abdominal respiration</td>
<td>• Absent Gular movements</td>
</tr>
</tbody>
</table>

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

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

5.3.8. The animal will remain anesthetized for 10–80 minutes, depending on the method and drug concentration used.

5.3.9. If supplemental anesthesia is needed, anesthetic solution can be dripped onto the animal’s skin to effect.

5.3.10. Monitor heart rate during anesthesia (e.g. direct observation [ventral midline, caudal to the shoulders], Electrocardiogram [ECG], Doppler flow detector).

**NOTE:** Normal values for heart rates are not well established in amphibians.

5.4. Post Anesthetic Care

5.4.1. General Considerations

5.4.1.1. Closely monitor fish/amphibians recovering from anesthesia until they are swimming/moving normally and have completely regained their righting response.

5.4.2. Fish

5.4.2.1. Place the fish in un-medicated water in a holding tank.
5.4.2.2. To speed recovery, create a flow of oxygenated water over the gills by either:

5.4.2.2.1. Gently moving the fish back and forth in the water or
5.4.2.2.2. Opening and closing the mouth several times.

5.4.3. Amphibians

5.4.3.1. After procedures are completed, thoroughly rinse the animal with fresh water.
5.4.3.2. Recovery chamber:

5.4.3.2.1. Aquatic Species – place animal in well-oxygenated, un-medicated water in a holding tank.
5.4.3.2.2. Terrestrial Species - place animal in a container lined with wet towels until fully recovered.

5.4.3.3. Do not raise the amphibian's body temperature above that of the species' normal range in an attempt to speed recovery.

6. PERSONNEL SAFETY

6.1. Medical Emergencies: CALL 911.
6.2. When working with animals wear appropriate PPE, observe proper hygiene, and be aware of allergy, zoonosis, and injury risks. Refer to the CARE Occupational Health and Safety webpage for more information.
6.3. MS-222 (tricaine methanesulfonate) safe practices:

6.3.1. Wear protective clothing, gloves, and goggles when handling MS-222 powder or animals exposed to MS-222.
6.3.2. If possible, work inside a fume hood to prepare a concentrated stock. Mix MS-222 powder in a volume of water appropriate to obtain the desired concentration and based on manufacturer’s recommendations. Wear gloves and use a utensil to stir until all powder is dissolved.
6.3.3. Dispose of MS-222 waste by flushing down the drain to a sanitary sewer with an excess of water. Do not discard MS-222 directly into surface water, storm water conveyances or catch basins.
6.3.4. If in a remote location where a sewer may not be readily available, further dilute the solution with water and dump wastes on land, in a location away from water.

7. ANIMAL RELATED CONTINGENCIES

7.1. Post contact information for emergency assistance in a conspicuous location within the animal facility.
7.2. Non-emergency veterinary questions and requests for care, email CARE veterinary staff at care@cornell.edu.
7.3. Emergency veterinary care is available at all times including after working hours and on weekends and holidays by calling the CARE pager (1-800-329-2456).
8. REFERENCES


8.5. CARE Occupational Health and Safety webpage: http://www.research.cornell.edu/care/OHS.html
9. **APPENDIX**

9.1. Immersion anesthetic agents used in fish and amphibian species

<table>
<thead>
<tr>
<th>Anesthetic Agent</th>
<th>Dose (mg/L of buffered aqueous solution)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS-222 (tricaine methanesulfonate)</td>
<td>75–125 (induction) and 50–75 (maintenance)</td>
<td>Buffer with equal weight of sodium bicarbonate This is the only FDA approved anesthetic for fish (21-day withdrawal).</td>
</tr>
<tr>
<td>Benzocaine hydrochloride</td>
<td>25–100</td>
<td>Small margin of safety between effective and lethal doses. Buffer solution with sodium bicarbonate to maintain neutral pH</td>
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<td>MS-222 (tricaine methanesulfonate)</td>
<td>250-500 mg/L of buffered aqueous solution 1-2 g/L of buffered aqueous solution 2-3 g/L of buffered aqueous solution</td>
<td>Tadpoles Frogs and salamanders Toads Buffer with equal weight of sodium bicarbonate</td>
</tr>
<tr>
<td>Benzocaine (powder or hydrochloride)</td>
<td>2 g/L of buffered aqueous solution</td>
<td>True toads, spadefoots, and large salamanders Buffer solution with sodium bicarbonate to maintain neutral pH</td>
</tr>
</tbody>
</table>

9.1.1. MS-222:

9.1.1.1. **Always buffer solution with an equal weight of sodium bicarbonate to maintain neutral pH**

9.1.1.2. In solution, MS-222 will lose efficacy if kept longer than 7 days.

9.1.1.3. MS-222 is a light-sensitive chemical and must be kept in a dark container or in a cabinet/drawer.

9.1.1.4. MS-22 has a wide margin of safety.
9.1.2. Benzocaine:

9.1.2.1. **Always buffer solution with sodium bicarbonate to maintain neutral pH**

9.1.2.2. Dissolve powder in appropriate solvent (e.g. water, or ethanol) to create a stock solution.

10. HISTORY

<table>
<thead>
<tr>
<th>Date</th>
<th>Event</th>
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<tbody>
<tr>
<td>03 NOV 15</td>
<td>New Format – Converted by: J. Kirby</td>
</tr>
<tr>
<td>01 OCT 15</td>
<td>Most Recent Annual Review – Reviewed by: Dr. D. Jeffery</td>
</tr>
<tr>
<td>31 JAN 04</td>
<td>New Issued – Original Author: Dr. J. Gourdon; Referee: G. Wooster</td>
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