**Animal Care & Use Committee (ACUC)**

**Guideline & Standard Operating Procedure**

**for**

**MS-222 (TMS, tricaine methane sulfonate)**

**Version: May 6, 2015**

UP 110 Fish and Amphibian Anesthesia

The intent of this Animal Care and Use Procedure (ACUP) is to describe common anesthesia procedures for fish and amphibians. This ACUP is intended for use by investigators and staff who have approval to anesthetize fish and amphibians in their study protocol. Any deviation must be approved by the IACUC prior to its implementation.

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**1. Introduction/Scope**

1. This document provides guidelines for fish and amphibian anesthesia procedures, safety, and post-anesthetic care and monitoring for the use of MS-222.
2. MS-222 or TMS or tricaine methane sulfonate is used at Cal Poly Pomona as an anesthetic and euthanasia agent in fish and amphibian species. As such, it poses two issues of concern to the ACUC: 1) its safe preparation and use by animal handlers and 2) not being of pharmaceutical grade.
3. MS-222 is an FDA approved fish anesthetic (FDA ANADA 200-226) (see the Western Chemical reference).
4. 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 (see the NIH reference).

**2. Materials**

1. Anesthetic agent [e.g. reagent or pharmaceutical grade tricaine methane sulfonate (MS-222) depending on availability], sodium bicarbonate (if using MS-222). Note: The 2011 edition of the “Guide for the Care and Use of Laboratory Animals” (pg. 31) discusses the preferred “use of pharmaceutical-grade chemicals to ensure that toxic or unwanted side effects are not introduced into studies conducted with experimental animals”. Thereby, this document was reviewed and adopted by the CPP ACUC. By virtue of that, the members of the ACUC accept the use of MS-222 in reagent grade form, acknowledging that no pharmaceutical grade product is available.
2. Chemical Gloves (e.g. Nitrile): When handling animals, wear non-powdered gloves pre-moistened with distilled or de-chlorinated water)
3. Transport, anesthetic and recovery tanks
4. Oxygenation equipment (e.g. air pump, tubing, and air stone)

**3. Procedures**

1. General considerations
2. 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.
3. 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).
4. Fish Anesthesia – Dosage 50 mg/L of water used
5. Fast fish for 12–24 hours prior to anesthesia; reduces fecal contamination and risk of regurgitation.
6. 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. Buffer water to a pH of 7.0 – 8.0 using sodium bicarbonate after addition of MS-222 and before addition of fish.
7. Maintain adequate oxygenation of holding tanks throughout induction, anesthesia, and recovery.

* Supply oxygen via air pump and air stone, or similar device

1. Maintain water temperature at the species’ normal optimum during both anesthesia and recovery.
2. Anesthesia is achieved by immersion in an anesthetic solution.
3. See Appendix for a partial list of agents and doses. vi. Stages of anesthesia in fish:

|  |  |  |
| --- | --- | --- |
| 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. |

1. Allow animal to reach appropriate level of anesthesia for planned procedures.
2. While performing procedures, keep the fish’s skin moist and the gills submerged or regularly flushed with well oxygenated water.
3. 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.
* Observe gill color; should be dark pink to light red.
* If respirations become extremely slow or stop, place the fish in anesthetic-free recovery water until respirations resume

1. Amphibian Anesthesia – dosage 0.05 – 0.2% (0.5g – 2.0 g per liter)
   * 1. Fast for 12–24 hours prior to anesthesia to decrease incidence of regurgitation
     2. 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 – 8.0 using sodium bicarbonate after addition of MS-222 and before addition of amphibian.
     3. Maintain temperature at the species’ normal optimum during both anesthesia and recovery.
     4. Anesthetic induction may produce an excitement phase. Induce anesthesia in a container that will preclude injury from the animal jumping or falling out.
     5. Anesthesia is achieved by immersion in an anesthetic solution. See Appendix for a partial list of agents and doses.

* Alternate methods can also be used if necessary. Contact CARE for assistance.
  + 1. 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.

* + 1. Allow animal to reach appropriate level of anesthesia for planned procedures.
    2. Remove the animal from the anesthetic bath after appropriate level of anesthesia is reached. Keep the amphibian’s skin moist throughout procedures.
    3. If supplemental anesthesia is needed, anesthetic solution can be dripped onto the animal’s skin.
    4. The animal will remain anesthetized for 10–80 minutes, depending on the method and drug concentration used.
    5. 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.

1. Post anesthetic Care
2. General consideration

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

1. 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

1. 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.

1. Fish Euthanasia
2. Dosage 250 mg/L
3. 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 – 8.0 using sodium bicarbonate after addition of MS-222 and before addition of the fish.
4. Secondary assurance of death - Rapid chilling: Submerge fish in 2-4⁰ C chilled water. Fish should not be in direct contact with ice. Fish must remain in the chilled water for 10 minutes following cessation of opercular movement.
5. Amphibian Euthanasia
   * 1. Dosage 0.5 – 1.0% (5 -10 g/L)
     2. 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 – 8.0 using sodium bicarbonate after addition of MS-222 and before addition of the amphibian.
     3. Secondary assurance of death - Rapid chilling: Submerge amphibian in 2-4⁰ C chilled water. amphibian should not be in direct contact with ice. amphibian must remain in the chilled water for 10 minutes following cessation of breathing.

**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).

**4. Personnel Safety**

* 1. Medical Emergencies: call 911
  2. It is the responsibility of the named PI on the animal study protocol to assure the safe use of MS-222 by laboratory staff. All personnel involved specifically with animals need to be named on the approved protocol, including documentation of their training both on the use of the animal and MS-222. The training should include review of the SDS (Safety Data Sheet).
  3. Laboratories should establish their own practices in handling MS-222 in the solid form. Questions regarding safety practices when using MS-222 should be directed to Environmental Health and Safety at 909.869.4697
  4. When working with animals wear appropriate PPE, observe proper hygiene, and be aware of allergy, zoonosis, and injury risks.
  5. MS-222 (tricaine methane sulfonate) safe practices:
     1. MS-222 is a powder and a respiratory irritant which can cause coughing and severe irritation of the upper airways in sensitive individuals. Wear protective clothing (e.g. Lab Coat), chemical resistant gloves (e.g. Nitrile), dust mask and goggles when handling MS-222 powder. If work is done in a fume hood the dust mask should not be necessary.
     2. If 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.
* Use a top loading balance with a clear plastic wind/breeze guard.
* Wear goggles, chemical resistant gloves (e.g. Nitrile), and lab Coat as PPE (personal protective equipment).
* Working under a fume hood can be problematic because MS-222 is a light powder and the air currents can blow dust particles around.

1. In typical use, the mode of action of MS-222 in solution is permeation of fish or amphibian skin. Of course, it can also permeate human skin (albeit at a much lower rate), particularly when dissolved. Personnel should wear chemical resistant gloves (e.g. Nitrile) when reaching into tanks containing MS-222 or handling exposed animals. Personnel should be aware of and attentive to nausea or headaches after prolonged exposure to even a 0.1% solution of MS- 222
2. Dispose of MS-222 wastes 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.

**5. Animal Related Contingencies**

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

**6. References**

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* Mitchell, M.A. 2009. Anesthetic Considerations for Amphibians. Journal of Exotic Pet Medicine, 18(1), 40-49.
* *CARE Occupational Health and Safety webpage.* [*http://www.research.cornell.edu/care/OHS.html*](http://www.research.cornell.edu/care/OHS.html)
* 8th Edition, Guide for the Care and Use of Laboratory Animals (2011), National Research Council, Washington, DC: <http://www.nap.edu/catalog.php?record_id=12910>
* <http://www.wchemical.com/tricaine-s-ms-222.html>
* <http://www.research.psu.edu/policies/research-protections/iacuc/iacuc-guideline-xxii>
* <http://www.orcbs.msu.edu/animal_handler/programs_guidelines/safety_guidelines/guidelines/ms222_safety_guideline_r2_2010.pdf>
* Safety training documentation forms are available on the EH&S Web Site at <http://www.csupomona.edu/~ehs/ftp/IIPPForms/Training.doc>
* AVMA 2013 Guidelines for Euthanasia: <https://www.avma.org/kb/policies/documents/euthanasia.pdf>
* National Institute of Health, “Retinal toxicity associated with occupational exposure to the fish anesthetic MS-222”: <http://www.ncbi.nlm.nih.gov/pubmed/9402834>