McGill

STANDARD OPERATING PROCEDURE #118

FISH AND AQUATIC AMPHIBIAN ANESTHESIA

1. PURPOSE

The intent of this Standard Operating Procedure (SOP) is to describe common anesthesia procedures for fish and aquatic amphibians.

2. RESPONSIBILITY

Principal investigator (PI) and their research staff, veterinary care staff.

3. MATERIALS

3.1. Anesthetic agent (MS-222, Benzocaine HCl, isoflurane or eugenol)

3.2. Gas induction chamber

3.3. Water-insoluble lubricant

3.4. Absorbent pad

3.5. Personal protective equipment: gloves, protective clothing (lab coat), eye protection

3.6. Fume hood

3.7. Holding and recovery tank

3.8. Air supply stone

4. FISH ANESTHESIA PROCEDURES

4.1. If using a new anesthetic protocol or species, anesthetize a few fish and follow them through full recovery to ensure drug dosages and techniques are safe and provide sufficient anesthetic depth for the intended procedures.

4.2. Fast fish for 12–24 hours prior to anesthesia. This reduces fecal contamination and risk of regurgitation.

4.3. Maintain adequate oxygenation:

4.3.1. Supply via an air supply stone or similar device.

4.3.2. Oxygenate all water chambers during anesthesia and recovery.

4.4. 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.

4.5. Maintain water temperature at the species' normal optimum during both anesthesia and recovery.

4.6. Anesthetic agents for fish:

ANESTHETIC AGENT	DOSE	COMMENTS
MS-222* (tricaine methanesulfonate)	75–125 mg/L (induction) and 50–75 mg/L (maintenance)	Sodium bicarbonate should be added to stock solution to maintain neutral pH. Only FDA-approved anesthetic for fish (21 day withdrawal).
Benzocaine hydrochloride	25–100 mg/L	Sodium bicarbonate may need to be added to stock solution to maintain neutral pH. Small margin of safety between effective and lethal doses.
Eugenol (clove oil)*	40-60 mg/L	Eugenol is diluted in ethanol, with a maximum of 10ml of ethanol per liter of immersion water.

* Light-sensitive chemical: should be kept in a dark container or in a cabinet/drawer

4.7. Anesthetic agents for zebrafish (*Danio rerio*) and other small, warm-water laboratory fish:

ANESTHETIC AGENT	DOSE	COMMENTS
MS-222* (tricaine methanesulfonate)	50 mg/L (sedation) 50–100 mg/L (light anesthesia) 150–200 mg/L (surgical anesthesia)	Sodium bicarbonate should be added to stock solution to maintain neutral pH. Also used to anesthetize larval zebrafish.
Benzocaine hydrochloride	25–100 mg/L (light anesthesia)	Sodium bicarbonate may need to be added to stock solution to maintain neutral pH. Small margin of safety between effective and lethal doses.
Eugenol (clove oil)*	2-5 mg/L (sedation) 60-100 mg/L (surgical anesthesia)	Eugenol is diluted in ethanol, with a maximum of 10ml of ethanol per liter of immersion water.

* Light-sensitive chemical: should be kept in a dark container or in a cabinet/drawer

4.8. Stages of anesthesia in fish:

STAGE 1 Deep sedation	STAGE 2 Deep narcosis	STAGE 3 Surgical anesthesia
Cessation of voluntary swimming Loss of response to stimuli.	Decreased muscle tone Equilibrium loss Appropriate level for fin and gill biopsies.	Slow respiration and heart rate Total loss of activity to stimuli.

4.9. Assess for surgical plane of anesthesia by monitoring:

4.9.1. Total loss of equilibrium and muscle tone

4.9.2. Decreased respiratory rate

4.9.3. No response to stimuli: firmly squeeze at the base of the tail to determine response to stimuli.

4.10. Evaluate respiratory rate and gill color:

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

4.10.2. Observe gill color: should be dark pink to light red.

4.10.3. If respirations become extremely slow or stop, place the fish in anesthetic-free recovery water until respirations resume.

5. AQUATIC AMPHIBIAN ANESTHESIA PROCEDURES

5.1. Anesthesia methods are achieved by:

5.1.1. Immersion in an anesthetic solution.

5.1.2. Placement in an anesthetic gas induction chamber.

5.1.3. Application of anesthetic preparations to the skin.

5.2. Fast for 12 to 24 hours prior to anesthesia to decrease incidence of regurgitation.

5.3. Keep amphibians moist during time out of water.

5.4. Induce anesthesia in a container that will prevent the animal jumping or falling out in order to avoid injury.

5.5. Anesthetic induction may produce an excitement phase.

5.6. Anesthetic Agents:

ANESTHETIC AGENT	DOSE	COMMENTS
MS-222* (tricaine methanesulfonate)	250-500 mg/L	Tadpoles
	1-2 g/L	Frogs and salamanders After immersion for 20 minutes, it provides surgical anesthesia for 30 min. (1g/L) to 60 min. (2g/L).
	2- 3 g/L	Toads
Benzocaine (powder or hydrochloride)	2 g/L	True toads, spadefoots, and large salamanders (see below)
Isoflurane	Variable	Can be applied to skin in an adhesive patch or viscous gel (see below)
Eugenol (clove oil)*	350ul/L	As eugenol is an oil, mixture should be thoroughly mixed before use.

* Light-sensitive chemical: should be kept in a dark container or in a cabinet/drawer

5.6.1. MS-222: Buffer solution with sodium bicarbonate to maintain neutral pH. Wide margin of safety.

5.6.2. Benzocaine: Dissolve powder in ethanol to create a stock solution. Buffer solution with sodium bicarbonate to maintain neutral pH.

5.6.3. Isoflurane administration options:

5.6.3.1. Mix into a viscous solution using a water-insoluble lubricant and water.

5.6.3.2. Inject into an absorbent pad and apply directly to the dorsum of the animal.

5.7. Pulmonary respiration will cease during anesthesia; therefore, respiratory rate cannot be used to monitor anesthetic depth; however, cutaneous respiration is sufficient to prevent clinical hypoxia during anesthesia.

5.8. Monitor heart rate during anesthesia by one of the following methods (Note: Normal values for heart rates have not been published):

5.8.1. Direct observation (ventral midline, caudal to the shoulders)

5.8.2. Electrocardiogram (ECG)

5.8.3. Ultrasonagraphy

5.8.4. Doppler flow detector

5.9. Stages of Anesthesia in Amphibians:

INDUCTION	LIGHT ANESTHESIA	SURGICAL ANESTHESIA
Decreased gular movement and diminished withdrawal reflex.	Loss of righting reflex and absence of abdominal respirations.	No withdrawal reflex (toe pinch) and gular movements cease.

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

5.11. Remove the animal from the anesthetic bath and rinse with fresh water, or remove the topical preparation by rinsing.

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

5.13. Determine full recovery from anesthesia by monitoring when the righting reflex returns and animal is able to move normally. It typically takes 30 to 90 minutes after the animal is rinsed with fresh water.

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

5.14.1. Increased body temperature will increase metabolism and oxygen requirements.

5.14.2. Cutaneous respiration may not be sufficient to maintain adequate oxygenation in this situation.

5.15. Do not apply alcohol or other preparations that contain alcohol directly to the skin of an amphibian as absorption of these products through the skin may dissolve normal secretions that protect the animal from dehydration and infections.

6. SAFETY PRACTICES

6.1. MS-222:

6.1.1. Wear protective clothing, gloves, and eye protection when handling the MS-222 powder.

6.1.2. Wear gloves to handle animals exposed to MS-222

6.1.3. Making MS-222 solutions:

6.1.3.1. Contact Environment Health and Safety Department for safe handling, use, and storage procedures.

6.1.4. Disposal of MS-222 waste:

6.1.4.1. Contact the Waste Management department for disposal procedures.

6.2. Eugenol:

6.2.1. Wear protective clothing, gloves and eye protection when handling eugenol.

6.2.2. Wear gloves to handle animals exposed to eugenol.

6.2.3. Making eugenol solutions:

6.2.3.1. Contact Environment Health and Safety Department for safe handling, use, and storage procedures.

6.2.4. Disposal of eugenol waste:

6.2.4.1. Contact the Waste Management department for disposal procedures.

6.2.5. Thoroughly wash hands after handling or administering eugenol.

SOP REVISION HISTORY

DATE	NEW VERSION
2021.01.13	5.6 Anesthetic Agents: Eugenol (clove oil)* Dose: ~~40-60mg/mL~~ **350ul/L** Comments: ~~Eugenol is diluted in ethanol, with a maximum of 10ml of ethanol per liter of immersion water.~~ As eugenol is an oil, mixture should be thoroughly mixed before use.
2021.11.03	**4.7. Anesthetic agents for zebrafish (Danio rerio) and other small, warm-water laboratory fish:** **MS-222* (tricaine methanesulfonate). Dose: 50 mg/L (sedation), 50–100 mg/L (light anesthesia), 150–200 mg/L (surgical anesthesia), Notes: Sodium bicarbonate should be added to stock solution to maintain neutral pH. Also used to anesthetize larval zebrafish.** **Benzocaine hydrochloride. Dose: 25–100 mg/L (light anesthesia), Notes: Sodium bicarbonate may need to be added to stock solution to maintain neutral pH. Small margin of safety between effective and lethal doses.** **Eugenol (clove oil)*. Dose: 2-5 mg/mL (sedation), 60-100 mg/L (surgical anesthesia). Notes: Eugenol is diluted in ethanol, with a maximum of 10ml of ethanol per liter of immersion water.**
2021.11.03	6.3.1. Wear protective clothing, gloves, and ~~goggles~~ **eye protection** when handling the MS-222 powder. **6.1.3.1. Contact Environmental Health and Safety Department for safe handling, use, and storage procedures..** ~~6.1.3.1. 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.Dilute the stock solution further as required.~~ ~~6.1.3.2. Wear gloves and use a utensil to stir until all powder is dissolved.~~
2021.11.03	6.1.4.1. ~~MS-222 should be collected and disposed of as chemical waste.~~ Contact the Waste Management department for ~~details~~ **disposal procedures**. ~~6.1.4.2. Do not discard MS-222 directly into sinks, drains, surface water, storm water conveyances or catch basins.~~
2021.11.03	**6.2.3.1. Contact Environmental Health and Safety Department for safe handling, use, and storage procedures.** ~~6.2.3.1. Work inside a fume hood to prepare a concentrated stock solution.~~ ~~6.2.3.2. Work areas should be protected from spills by placing an absorbent pad with absorbent material facing up.~~ ~~6.2.3.3. Dilute the stock solution further as required.~~
2021.11.03	6.2.4.1. ~~Eugenol should be collected and disposed of as chemical waste.~~ Contact the Waste Management department for ~~details~~ **disposal procedures**. ~~6.2.4.2. Do not discard eugenol directly into sinks, drains, surface water, storm water conveyances or catch basins~~.
2022.01.13	3.5. Personal protective equipment: gloves, protective clothing (lab coat), ~~goggles~~ **eye protection**
2022.01.13	6.2.1. Wear protective clothing, gloves and ~~goggles~~ **eye protection** when handling eugenol.