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, veterinarians, veterinary care staff.

4. FISH ANESTHESIA PROCEDURES

4.1. If using a new anesthetic protocol or species, anesthetize a few fish and follow 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.

4.4. Maintain adequate oxygenation:

4.7. 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

5. AQUATIC AMPHIBIAN ANESTHESIA PROCEDURES

5.1. Anesthesia methods are achieved by:

5.1.1. Immersion in an anesthetic solution.

5.1.2. 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, and until the animal recovers,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.

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. s1k nBT1**9**.68 6**9**72-Tm( )T**2**/**9** (.)2.3 nocS