



Cornell University

Cornell Center for Animal Resources and Education

CARE110.01 Fish and Aquatic Amphibian Anesthesia

The intent of this standard operating procedure (SOP) is to describe common anesthesia procedures for fish and aquatic amphibians. This SOP is intended for use by investigators and staff who have approval to anesthetize fish and aquatic amphibians in their IACUC-approved studies. This SOP is approved by the Cornell Institutional Animal Care and Use Committee (IACUC) and the Cornell Center for Animal Resources and Education (CARE). Any exemption must be approved by the IACUC prior to its application.

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1. Introduction

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

2. Materials

- Anesthetic agent (MS-222, Benzocaine HCl or powder)
- Gloves
- Fume hood
- Anesthetic and recovery tank
- Air supply stone

3. Procedures

A) Fish Anesthesia

Precautionary Note: 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.

- i) Fast fish for 12–24 hours prior to anesthesia
 - Note:** Reduces fecal contamination and risk of regurgitation
- ii) Maintain adequate oxygenation
 - (1) Supply via an air supply stone or similar device
 - (2) Oxygenate all water chambers during anesthesia and recovery
- iii) Use water taken from original fish holding tank for transport, anesthetic and recovery chambers
 - Note:** If using another water source closely duplicate the water quality parameters (i.e., chlorine, temperature, pH and ammonia) of the original holding tank
- iv) Maintain water temperature at the species' normal optimum during both anesthesia and recovery
- v) Anesthesia achieved by immersion in an anesthetic solution:

Anesthetic agent	Dose (mg/L of aqueous solution)	Comments
MS-222 (tricaine methanesulfonate)	75–125 (induction) and 50–75 (maintenance)	Sodium bicarbonate must be added to stock solution to maintain neutral pH. Only FDA approved anesthetic for fish (21 day withdrawal).
Benzocaine hydrochloride	25–100	Sodium bicarbonate may need to be added to stock solution to maintain neutral pH. Small margin of safety between effective and lethal doses.

- (1) MS-222:
 - (a) Buffer solution with sodium bicarbonate to maintain neutral pH
 - (b) In solution, MS-222 will lose efficacy if kept longer than 7 days
 - (c) MS-222 is a light-sensitive chemical, it must be kept in a dark container or in a cabinet/drawer
 - (d) Wide margin of safety

(2) Benzocaine:

(a) Dissolve powder in ethanol to create a stock solution

(b) Buffer solution with sodium bicarbonate to maintain neutral pH

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 activity to stimuli.

vii) Assess for surgical plane of anesthesia by monitoring:

(1) Total loss of equilibrium and muscle tone

(2) Decreased respiratory rate

(3) No response to stimuli; firmly squeeze at the base of the tail to determine response to stimuli

viii) Evaluate respiratory rate and gill color:

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

(2) Observe gill color; should be dark pink to light red

(3) If respirations become extremely slow or stop, place the fish in anesthetic-free recovery water until respirations resume

B) Aquatic Amphibian Anesthesia

i) Fast for 12–24 hours prior to anesthesia to decrease incidence of regurgitation

ii) Use water taken from original holding tank for transport, anesthetic and recovery chambers

Note: If using another water source closely duplicate the water quality parameters (i.e., chlorine, temperature, pH and ammonia) of the original holding tank

iii) Maintain water temperature at the species' normal optimum during both anesthesia and recovery

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

v) Anesthetic induction may produce an excitement phase

vi) Anesthesia achieved by Immersion in an anesthetic solution

Anesthetic Agent	Dose	Comments
MS-222 (tricaine methanesulfonate)	250-500 mg/L of aqueous solution	Tadpoles
	1-2 g/L of aqueous solution	Frogs and salamanders
	2-3 g/L of aqueous solution	Toads
Benzocaine (powder or hydrochloride)	2 g/L of aqueous solution	True toads, spadefoots, and large salamanders (see below)

(1) MS-222:

- (a) Buffer solution with sodium bicarbonate to maintain neutral pH
- (b) In solution, MS-222 will lose efficacy if kept longer than 7 days
- (c) MS-222 is a light-sensitive chemical, it must be kept in a dark container or in a cabinet/drawer
- (d) Wide margin of safety

(2) Benzocaine:

- (a) Dissolve powder in ethanol to create a stock solution
- (b) Buffer solution with sodium bicarbonate to maintain neutral pH

vii) Keep amphibians moist during time out of water

Note: pulmonary respiration will cease during anesthesia; therefore, respiratory rate can not be used to monitor anesthetic depth; however, cutaneous respiration is sufficient to prevent clinical hypoxia during anesthesia

viii) Monitor heart rate during anesthesia:

- (1) Direct observation (ventral midline, caudal to the shoulders)
- (2) ECG
- (3) Ultrasonography
- (4) Doppler flow detector

Note: Normal values for heart rates have not been published

ix) 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.

x) Allow animal to reach appropriate level of anesthesia for planned procedures

xi) Remove the animal from the anesthetic bath and rinse with fresh water

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

xii) Determine full recovery from anesthesia by monitoring when the righting reflex returns and animal is able to move normally

Note: It typically takes 30–90 minutes after the animal is rinsed with fresh water.

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

(1) Increased body temperature will increase metabolism and oxygen requirements.

(2) Cutaneous respiration may not be sufficient to maintain adequate oxygenation in this situation.

xiv) 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.

C) Postanesthetic Care

i) Fish/amphibian should be placed in untreated water in a holding tank for recovery

ii) Fish/amphibian should be monitored frequently during recovery from anesthesia until they are swimming/moving normally and have completely regained their righting response

4. Safety

A) Working with MS-222

- i) Wear protective clothing, gloves and goggles when handling the MS-222 powder
- ii) Making MS-222 Solutions:
 - (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
 - (2) Dilute the stock solution further as required
 - (3) Wear gloves and use a utensil to stir until all powder is dissolved
- iii) Wear gloves to handle animals exposed to MS-222
- iv) Disposal of MS-222 waste:
 - (1) Flush down the drain to a sanitary sewer with an excess of water.
 - (2) 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

Note: Do not discard MS-222 directly into surface water, storm water conveyances or catch basins

B) General

- i) Zoonosis: refer to CARE reference below
- ii) Animal related injury: refer to CARE SOP 707.01 Animal Related Injury

5. References

- i) Standard Operating Procedures from Animal Resource Program, Penn State University; <http://www.research.psu.edu/orp/areas/animals/arp.asp> (accessed October 23, 2005)
- ii) Kohn, D.F., Benson, G.J., Wixson, S.K., White, W.J., Anesthesia and Analgesia in Laboratory Animals; Academic Press, New York, 1997; Chapter 15.
- iii) CARE SOP 707.01 Animal Related Injury: <http://www.research.cornell.edu/care/documents/SOPs/CARE707.pdf>
- iv) Zoonosis: <http://www.research.cornell.edu/care/zoonoses.html>

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