



Document: ACUP306.02	Issue Date: 04 OCT 18; Effective Date 04 OCT 18
Authorization: Dr. N. Place, IACUC Chair	Author: Dr. T. Pavek (Revision)

## FISH AND AMPHIBIAN EUTHANASIA

### 1. PURPOSE

- 1.1. The purpose of this Animal Care and Use Procedure (ACUP) is to describe the acceptable methods for euthanasia of fish and amphibians. This ACUP is approved by the Cornell Institutional Animal Care and Use Committee (IACUC). Any deviation must be approved by the IACUC prior to its implementation.

### 2. SCOPE

- 2.1. This ACUP is intended for trained personnel that will perform euthanasia on fish or amphibians.

### 3. INTRODUCTION

- 3.1. This ACUP defines the materials and methods approved for euthanizing fish and amphibians under protocol use at Cornell University. Contact Center for Animal Resources and Education (CARE) at [care@cornell.edu](mailto:care@cornell.edu) for more information or for other assistance.

### 4. MATERIALS AND EQUIPMENT

- 4.1. Materials and equipment are dependent upon method used.
  - 4.1.1. Topical euthanasia agent (e.g., tricaine methanesulfonate [MS-222]).
  - 4.1.2. Mechanical euthanasia tool (e.g., ice & bucket, guillotine, pithing tool)

### 5. PROCEDURE

**NOTE:** Prior to disposing of euthanized animals: for amphibians, verify the animal is dead by performing a physical method of euthanasia or by assuring the heart has stopped beating; for fish, perform a physical means of euthanasia or observe that opercular movement has ceased for at least 10 minutes.

## 5.1. External / Topical Chemical Agents

### 5.1.1. Tricaine methanesulfonate (MS-222):

5.1.1.1. MS-222 is acidic in solution and must be buffered by adding an equal weight of sodium bicarbonate or titrating to pH=7.0-7.5

5.1.1.2. Tank immersion method (fish or amphibians):

5.1.1.2.1. Place the animal in a buffered solution of MS-222, at a concentration of:

5.1.1.2.1.1. Fish: minimum concentration of 250 mg/l (species dependent\*);

5.1.1.2.1.2. Amphibian: average range of 2-3 g/l (most species)

5.1.1.2.2. Immerse until death results as defined by the NOTE above in section 5. Time to effect is proportional to MS-222 solution concentration.

**NOTE:** \*It is important to research the species of fish being euthanized for established MS-222 euthanasia dosing.

5.1.1.3. Alternative method (fish only):

5.1.1.3.1. Remove fish from water and flush gills with a buffered, concentrated solution of MS-222 (>250 mg/L).

### 5.1.2. Benzocaine hydrochloride (fish or amphibians):

5.1.2.1. Benzocaine-HCl is acidic in solution and must be buffered by adding an equal weight of sodium bicarbonate or titrating to pH=7.0-7.5.

5.1.2.2. Place animal into a bath of benzocaine hydrochloride solution of  $\geq 250$  mg/L; immerse until death results as defined by the **NOTE** at the beginning of section 5.

### 5.1.3. 2-phenoxyethanol (fish only):

5.1.3.1. Place fish into a bath of 2-phenoxyethanol solution at a concentration of 0.5–0.6 mL/L or 0.3–0.4 mg/L; immerse until death results.

**NOTE:** Chemical methods of euthanasia may require up to 3 hours before death results. A physical method, as per section 3.b, can be applied once unconsciousness is achieved.

## 5.2. Physical Methods

**NOTE:** Anesthesia or heavy sedation must be applied prior to the use of physical techniques unless otherwise approved by the IACUC.

**5.2.1. Decapitation (fish or amphibian):**

**5.2.1.1.** Use sharp equipment of the appropriate size for the species to be euthanized to ensure that the head is separated from the body rapidly and completely. Follow decapitation with pithing the brain via the open spinal canal.

**5.2.2. Pithing without decapitation (amphibians only):**

**5.2.2.1.** Flex the neck, identify the foramen magnum, insert a rigid metal rod cranially, and pivot/rotate the rod within the cranium to destroy the proximal spinal cord and brain.

**5.2.3. Thermal shock (only for specimens that are <4cm in total body length):**

**5.2.3.1. Rapid freezing:**

**5.2.3.1.1.** Anesthetize animal as per [ACUP 110 Fish and Amphibian Anesthesia](#) and then immerse in liquid nitrogen.

**5.2.3.2. Rapid Chilling (hypothermic shock) in tropical fresh water fish:**

**5.2.3.2.1.** Set up an ice bucket or cooler with ice slush.

**5.2.3.2.2.** Form a depression in the ice to expose water and prevent direct animal contact with the ice.

**5.2.3.2.3.** Pour the anesthetized fish and/or larvae into the depression.

**5.2.3.2.4.** Use the minimal amount of water for transfer of animals into ice water to prevent local warming of ice water by adding room temperature water to the ice.

**5.2.3.2.5.** Use a net for adults and a minimal volume of embryo medium for embryos / larvae.

**NOTE:** Rapid chilling is not effective for zebrafish embryos <3 days post fertilization (dpf).

**5.2.3.2.6.** Adult zebrafish should be exposed for a minimum of 10 minutes and fry 4 to 7 dpf for at least 20 minutes following loss of operculum movement.

## **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](mailto: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

- 8.1. NIH Guidelines for use of zebrafish:  
<http://oacu.od.nih.gov/ARAC/documents/Zebrafish.pdf>
- 8.2. Evaluation of rapid cooling and tricaine methanesulfonate (MS222) as methods of euthanasia in zebrafish (*Danio rerio*). JM Wilson, et al. JAALAS. 2009. Nov; 48(6), 785-789.
- 8.3. Neiffer, D.L., Stamper, M.A. 2009. Fish sedation, anesthesia, analgesia, and euthanasia: Considerations, methods, and types of drugs. *ILAR Journal*, 50(4), 343-360.
- 8.4. AVMA Guidelines on Euthanasia, June 2013:  
<https://www.avma.org/KB/Policies/Pages/Euthanasia-Guidelines.aspx>
- 8.5. Guidelines for Euthanasia of Nondomestic Animals, American Association of Zoo Veterinarians; 2006.
- 8.6. Laboratory Animals: Recommendations for euthanasia of experimental animals. Royal Society of Medicine Press, London, U.K. 1996.
- 8.7. Guidelines for the Use of Fishes in Research (2004), Copyright 2004 by the American Fisheries Society:  
[http://fisheries.org/docs/policy\\_useoffishes.pdf](http://fisheries.org/docs/policy_useoffishes.pdf)
- 8.8. Amphibian Medicine and Captive Husbandry, KM Wright & BR Whitaker, 2001; Krieger Publishing Company, Malabar, FL.
- 8.9. Guidance on the housing and care of the African clawed frog *Xenopus laevis*, BT Reed.
- 8.10. Formulary for Laboratory Animals. CT Hawk, et al. 3rd ed., 2005
- 8.11. CARE Occupational Health and Safety webpage:  
<http://ras.research.cornell.edu/care/OHS.html>

## 9. APPENDIX

- 9.1. None

## 10. HISTORY

Date:	Event:
04 OCT 18	Most Recent Annual Review – Reviewed by: Dr. E. Feldman
03 NOV 15	New Format – Converted by: J. Kirby
UNK 11	Revised – Revision Author: Dr. T. Pavek; Referee: Dr. P. Bowser
UNK 05	New Issued – Original Author: C. Brown

ORIGINAL COPY