



Cornell University

Cornell Center for Animal Resources and Education

CARE306.01 Fish and Amphibian Euthanasia

The intent of this procedure is to describe the acceptable methods of euthanasia of fish and amphibians. This SOP is intended for any personnel that will perform euthanasia on fish or amphibians. This procedure 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 submitted for approval to the IACUC prior to its application.

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1. Requirements

Ensure that all individuals responsible for euthanasia:

- a. Receive appropriate training to perform the procedure.
- b. Adhere to IACUC-approved protocols and institutional policies.
- c. Are knowledgeable in species differences in their metabolism, respiration, and tolerance to cerebral hypoxia.

2. Non-Physical Methods

- a. Injectable agents
 - i. Inject Sodium pentobarbital IP or IV into the fish or amphibian at a dose of 60 to 100 mg/ kg of body weight.
 - ii. Important: Verify that an animal is dead before disposing of the carcass by monitoring for respiratory or opercular movement.

Note: Time to effect may vary, with death occurring in up to 30 minutes.

- iii. Follow the injection with a physical method of euthanasia, if needed, to ensure death. Examples of physical methods include pithing and decapitation.
- b. External or topical agents

Tricaine methane sulfonate (TMS/MS222): place fish in a solution of MS222 dissolved in water (minimum concentration of 250 mg/L) until death is achieved.

Note: To ensure death a physical method of euthanasia such as pithing or decapitation may be used after the anesthetic bath.

i. Alternative methods:

- 1) Remove fish from water and flush gills with a concentrated solution of MS222 (>250 mg/L).
- 2) Inject MS222 into the lymph spaces or pleuroperitoneal cavities.

Note: MS222 is acidic and in concentrations >500 mg/L, it should be buffered with sodium bicarbonate to saturation resulting in a solution pH of 7.0–7.5.

ii. Benzocaine hydrochloride: Place fish into a bath of benzocaine hydrochloride solution of >250 mg/L.

iii. 2-phenoxyethanol: 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.

c. Inhalant agents

i. CO₂: Place fish or aquatic amphibian in a closed container. Displace oxygen by adding carbon dioxide from a compressed air cylinder, bubbling the CO₂ vigorously for 30 seconds, then observing for 10 minutes following cessation of breathing.

ii. Inhalant anesthesia: Euthanize fish by extended induction of inhalant anesthesia. Do **not** use this method to euthanize amphibians because of their ability to hold their breath for and survive long periods of anoxia.

Note: All inhalant agents require long exposure times to achieve death. If necessary, inhalants may be followed with a physical method of euthanasia after loss of consciousness.

3. Physical Methods

Important: Anesthesia or sedation must be applied prior to the use of physical techniques unless scientifically justified by user and approved by the IACUC.

a. Decapitation

Use sharp equipment of the appropriate size for the species to be euthanized to ensure that the head is quickly separated from the body rapidly and completely. Follow decapitation with pithing.

b. Pithing

Insert a rigid metal rod into the foramen magnum which is identified by the slight midline skin depression posterior to the eyes when the neck

is flexed. Ensure that both the brain and the proximal end of the spinal cord are destroyed. Follow pithing with decapitation.

c. Thermal shock (freezing)

Note: This method is controversial, as some believe that as ice crystals are formed, the animal will feel pain; however, in small fish (e.g., zebrafish) it appears that they die rapidly before the ice crystals form.

- i. Rapid freezing: Euthanize small fish, less than 10 cm in length, instantly by immersion in liquid nitrogen.
- ii. Slow freezing (thermal shock):
 - a. Option 1: Place fish in a container of system water and place in the freezer; as the temperature drops, the fishes' metabolism slows, and the fish sink to the bottom of the container
 - b. Option 2 (**From ZIRC): Set up an ice bucket or cooler with ice slush; form a depression in the ice to expose water; pour fish and larvae into the depression; use the minimal amount of water needed that will still prevent direct contact with ice.

4. Safety

MS-222 (tricaine methane sulphonate) safe practices:

- a. Wear protective clothing, gloves and goggles when handling the MS-222 powder.
- b. 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.
- c. Dilute the stock solution further as required.
- d. Wear gloves and use a utensil to stir until all powder is dissolved.
- e. Wear gloves to handle animals exposed to MS-222.
- f. Dispose of MS-222 wastes by flushing down the drain to a sanitary sewer with an excess of water.
- g. 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.
- h. Do not discard MS-222 directly into surface water, storm water conveyances or catch basins.

5. References

- a. *2000 Report of the AVMA Panel on Euthanasia*; JAVMA. Vol. 218, No. 5, March 1, 2001
<http://www.avma.org/resources/euthanasia.pdf>
- b. *Laboratory Animals: Recommendations for euthanasia of experimental animals*. Royal Society of Medicine Press, London, U.K. 1996.

- c. *Guidelines for the Use of Fishes in Research* (2004), Copyright 2004 by the American Fisheries Society.
 - d. http://www.fisheries.org/html/Public_Affairs/Sound_Science/Guidelines2004.shtml#Euth
 - e. ***Personal communication: Zoltán Varga, PhD, Director, Zebrafish International Resource Center (ZIRC)*
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Written by/date :
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April 17, 2003

Effective date :
May 2005

Review date :
August 2006

Referee:
P. Bowser

SOP :
CARE306.01