1. Purpose

This document details the procedures for humane euthanasia of Xenopus frogs (*Xenopus laevis and tropicalis*) and zebrafish (*Danio rerio*).

1. Scope

This policy applies to all persons performing euthanasia on aquatic species.

1. General Information

The contents of this document conform to the AVMA Guidelines for the Euthanasia of Animals 2020 edition.

1. Equipment and Supplies

MS 222 = Tricaine Methanesulfonate

Buffer Solution; sodium bicarbonate

Method of waste MS 222 disposal as chemical waste

Ice

Sodium hypochlorite or calcium hypochlorite solution

Other equipment as needed

1. Safety Requirements
   1. Personnel working with the powdered form of MS 222 will work in a fume hood or wear a face mask and safety glasses when weighing the powdered form or mixing the powdered form into solution. Gloves will be worn when handling these agents and any animal treated with these agents. The container used to store the solutions will be labeled with an expiration date (no more than 6 months beyond the mix date).
   2. MS222 must be disposed of as a chemical hazard. Collect any liquid or powder waste into a chemical hazard container, complete and attach a work tag and contact safety and risk management and/or fill out the form at <https://www.montana.edu/srm/forms/waste/> .
2. Zebrafish: Acceptable Methods of Euthanasia
3. Rapid Chilling (Hypothermic Shock)

Submerge fish in 2-4°C chilled water. Fish must not be in direct contact with ice.

1. Immersion times:
2. Adults: a minimum of 10 additional minutes following the loss of opercular movements or followed by decapitation once opercular movement ceases to confirm death.
3. Zebrafish fry 4 to 14 days post fertilization (dpf): at least 20 additional minutes following a loss of opercular movements or should be decapitated once opercular movement ceases to confirm death.
4. Not effective for zebrafish embryos (0-3 dpf)
5. Sodium hypochlorite or calcium hypochlorite solution
6. Zebrafish embryos (0-3 dpf): immerse is a dilute (10%) sodium hypochlorite solution.
7. MS 222

Tricaine Methanesulfonate (MS 222) is a benzoic acid derivative and the solution should be buffered with sodium bicarbonate. For zebrafish, a 20x stock solution can be made containing 10 g/L MS 222 and 20 g/L sodium bicarbonate, resulting in a pH between 7.0 and 7.5 for the solution. The stock solution should be protected from light and refrigerated or frozen. The solution should be replaced monthly and whenever a brown color is observed. Potency is increased in warm water and decreased in cold water.

Immersion in 0.25 g/L - 0.5 g/L MS 222 buffered with sodium bicarbonate for a pH between 7-7.5 for at least 30 minutes following cessation of opercular movement.

1. Effective for zebrafish 15 dpf – adult.
2. Effective for zebrafish 4 dpf – 14 dpf but must be followed by a secondary euthanasia method to confirm death. Secondary euthanasia methods include decapitation or immersion in a dilute (10%) sodium hypochlorite or calcium hypochlorite solution.
3. Not effective for zebrafish embryos (0-3 dpf). Use sodium hypochlorite or calcium hypochlorite solution.
4. Xenopus: Acceptable Methods of Euthanasia
5. Benzocaine

Benzocaine (182 mg/kg) applied topically to the ventrum - equivalent to 2.0 cm x 1 mm application of 20% gel. Apply gel then return frog to a wet bucket without water until deep anesthesia is confirmed (approximately 7 minutes). Wait 5 hours or apply secondary method of euthanasia (rapid freezing, double pithing, removing the heart, or decapitation) after 10 minutes.

1. MS 222

Refer to information on the use of MS 222 in section 6C above.

Immersion in 5 g/L MS 222 buffered with sodium bicarbonate for a pH between 7-7.5 for at least 1 hour following cessation of movement.

1. References

AVMA Guidelines for the Euthanasia of Animals, 2020 Edition. Pgs. 64 & 92 www.avma.org

Wallace CK et al. (2018). Effectiveness of Rapid Cooling as a Method of Euthanasia for Young Zebrafish (Danio rerio). JAALAS 57(1):58-63.

Torreilles, S. and D. McClure, S. Green. (2009). Evaluation and Refinement of Euthanasia methods for Xenopus laevis. JAALAS 48(5): 512-516