AUP Section: Animal Disposition
Species: Zebrafish

Methods of euthanasia: (select all that apply)

Parameters monitored: Loss of orientation, opercular movement

Primary methods:

Agent/Method Name	Dosage (in mg/kg if possible)	Route
Buffered Tricaine methanesulfonate (MS-222)	200-300 mg/L (0.2%) –buffer to a neutral pH	Immersion
Ice water	5 parts ice/1 part water, 2-4º C	Immersion

Secondary methods:

Agent/Method Name	Dosage (in mg/kg if possible)	Route
Freezing	Not applicable	Not applicable
Maceration (for non-transgenic)	Not applicable	Not applicable

Description of Euthanasia Procedure: (select all that apply):

Procedure	Description
Tricaine methanesulfonate	Euthanasia will be carried out as described in the most current version of the AVMA Guidelines for the Euthanasia of Animals Procedural Steps: 1. Immerse fish in a solution of tricaine methanesulfonate (Finquel or Tricaine-S). 2. The solution should be buffered with sodium bicarbonate to a pH of 7.0-7.5. 3. Depending on age, fish must remain in the solution for a period following cessation of opercular (gill) movement or heartbeat (larvae): a. Fish ≥8dpf: at least 10 minutes b. Fish <8dpf: at least 20 minutes 4. <3 dpf: dilute bleach solution (1-part sodium hypochlorite 6.15% to 5 parts water) should be added to the water for 5 minutes to ensure embryonic lethality.

Ice water	Euthanasia will be carried out as described in the most current version of the AVMA Guidelines for the Euthanasia of Animals Procedural Steps: 1. Immobilization by submersion in ice water (5 parts ice/1-part water, 2-4º C) 2. Depending on age, fish must remain in the solution for a period following cessation of opercular (gill) movement or heartbeat (larvae): a. Fish ≥8dpf: at least 10 minutes b. Fish <8dpf: at least 20 minutes

Details for Carcass Disposal:

Ova and embryos will be collected in a sieve and prevented from going down the drain. All carcasses and reproductive material will be frozen for incineration or another approved disposal method by OLAC.

References:

American Veterinary Medical Association. 2020. AVMA Guidelines on Euthanasia, 2020 update.

Guidelines for Use of Zebrafish in the NIH Intramural Research Program.

http://oacu.od.nih.gov/arac/documents/zebrafish.pdf

Strykowski JL and Schech JM. (2015) Effectiveness of Recommended Euthanasia Methods in Larval Zebrafish (Danio Rerio). JAALAS 54, 76-79.

University of Oregon (2008) Final Report to OLAW on Euthanasia of Zebrafish.

Wilson et al. (2009) Evaluation of rapid cooling and tricaine methanesulfonate (MS222) as methods of euthanasia in zebrafish (Danio rerio). JAALAS 48, 785-9.

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