

**University of Colorado Boulder
Office of Research Integrity
Institutional Animal Care and Use Committee
SOP # 29**

Euthanasia with tricaine methane sulfonate (MS 222)

PURPOSE

The purpose of this SOP is to standardize the practices of using buffered tricaine methane sulfonate (TMS, MS 222) for euthanasia of aquatic animals in research at CU Boulder and research sponsored by CU Boulder. Euthanasia is the act of inducing humane death in an animal by a method that induces rapid loss of consciousness and death with a minimum of pain, discomfort or distress.

GENERAL PROCEDURES

- Available as tricaine methane sulfonate (TMS), MS 222 can be used for euthanasia of amphibians and fish.
- Tricaine is a benzoic acid derivative and must be buffered with sodium bicarbonate.
- A 10 g/L stock solution can be made, and sodium bicarbonate added to saturation, resulting in a pH between 7.0 and 7.5 for the solution. Check the pH of your stock solution. Store the stock solution in a dark brown bottle, and keep refrigerated or frozen if possible. Replace the solution monthly and any time a brown color is observed.
- To get an MS 222 concentration of 250 mg/L from the stock solution of 10 g/L, add 1 part stock solution to 39 parts water.
- Due to species differences in response to MS 222, a secondary method of euthanasia is recommended in some finfish and amphibians to ensure death.
- MS 222, once mixed, is a hazardous chemical and must be disposed of through Environmental Health & Safety.

EUTHANASIA PROCEDURES FOR FINFISH AND AMPHIBIANS

For euthanasia, a concentration of ≥ 250 mg/L (MS 222) is recommended:

- Fish must be left in this solution for at least 10 minutes following cessation of opercular movement. Large fish may be removed from the water, a gill cover lifted, and a concentrated solution from a syringe flushed over the gills (>250 mg/L)*.
- Amphibians must be left in this solution for at least 10 minutes following cessation of movement.

EUTHANASIA PROCEDURES FOR ZEBRAFISH ≥ 8 dpf **

- The concentration of MS 222 for euthanasia of zebrafish is 200-300 mg/L
- Zebrafish must be left in this solution for at least 10 minutes following cessation of opercular movement.
- For zebrafish between 0 and 15 dpf**, use of MS 222 for euthanasia must be followed up with a secondary method of euthanasia. The IACUC recommends the following options as secondary methods of euthanasia for aquatics:
 - Pithing (spinal column)
 - Decapitation
 - Removal of multiple organs for tissue procurement
 - Exsanguination
 - Addition of sodium hypochlorite to the solution (for zebrafish between 0 and 7 dpf)†

REFERENCES

- †AVMA Guidelines for the Euthanasia of Animals 2013 Edition
- Duke University & Duke University Medical Center Animal Care and Use Program Policy on Euthanasia Version Approved by the IACUC 23 August 2012 http://vetmed.duhs.duke.edu/PDF/Policies/Animal%20Use%20Policies/policy_on_euthanasia.pdf
- *Cornell University ACUP 306 Fish and Amphibian Euthanasia <http://www.research.cornell.edu/care/documents/ACUPs/ACUP306.pdf>
- ** Guidelines for Use of Zebrafish in the NIH Intramural Research Program, revised 5/8/2013 <http://oacu.od.nih.gov/ARAC/documents/Zebrafish.pdf>