UNIVERSITY OF WASHINGTON
Veterinary Services Reference Sheet

Use of Eugenol (from Sigma) for Anesthesia/Euthanasia in Finfish Species – August 2013

Stock Solution Preparation Method:

Standardized and known concentrations of the essential oil must be used for accurate dosing.

> Recommended Eugenol source and product:
> Sigma-Aldrich: **E51791 Aldrich** Eugenol *ReagentPlus*®, 99%
> Sizes availability - 5, 100, 500 g in glass bottle
> http://www.sigmaaldrich.com/catalog/product/aldrich/e51791?lang=en&region=US

Eugenol is poorly soluble in water. Is should be diluted 1:10 with 95% ethanol (1 part Eugenol to 9 parts ethanol) to yield a working solution of 100 mg/ml (each ml of Sigma E51791 will contain 1.067 g of Eugenol). Stock solution must be made inside of a chemical fume hood with personnel wearing a lab coat, double nitrile gloves, and safety glasses. The final stock solution must be dated when created and stored in a dark colored bottle at room temperature. This solution is good for one month after creation and should be labeled with an expiration date or an SOP reflecting this expiration date needs to be in place.

Disposal of Stock Solutions:

Solutions of Eugenol of 100 mg/ml or greater are considered to be hazardous waste. Therefore waste stock solutions must be collected for EH&S Environmental Programs pickup and disposal as hazardous waste.

To ensure proper disposal, use the "Waste Evaluation Form" available at the link below: http://www.ehs.washington.edu/forms/epo/1957.pdf. Complete it and fax to John Wallace at 206-685-2915, or it can be mailed to UW Box 354110.

Use the "Waste Collection Request Form" at the link below to schedule EH&S to come and pick up your unused or used Eugenol solutions: http://www.ehs.washington.edu/forms/epo/1470.pdf

Potential Hazard/Warning Associated with use:

Concentrated forms of Eugenol are a sensitizer and can be corrosive to the skin and eyes (humans). High concentrations of Eugenol dissolved in water are irritating and contact with eyes and mucous membranes should be avoided (fish).

Sedation/Anesthesia/Euthanasia Dosage Ranges:

Dosage ranges will also vary with fish size, type, and environment from which the fish species originates. It is highly recommended that before use on the general fish population all dosages of Eugenol should be first tested on a sub-population to evaluate anesthetic efficacy and depth (e.g. required level of anesthesia reached and maintained) and the potential for adverse effects (e.g. prolonged recovery from anesthesia or lack of recovery from anesthesia).

Sedation dose range – 0.06 – 0.10 ml of stock solution added to 1 L of water (6 – 10 mg/L)

Anesthesia dose – 0.4 – 1.2 ml of stock solution added to 1 L of water (40 – 120 mg/L)

Note: Fish anesthetized with eugenol cannot be released into the wild or used for human or animal consumption. Carcasses must be disposed of according to FDA guidelines (e.g. medical waste disposal or sanitary landfill.). Washington State (local) composting companies will not accept fish carcasses exposed to any chemical anesthesia/euthanasia agents.

Euthanasia dose – 1.5 ml of stock solution added to 1 L of water (150 mg/L)

Note: Fish must remain in euthanasia solution for 10 minutes after last opercular movement is observed, to ensure death of fish either a large vessel should be severed (gill arch or tail vein) or the carcass should be frozen.

References:

1. FDC-CVM Issues Guidance on Use of Clove Oil and Eugenol for Fish http://www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm134765.htm
2. AVMA Guidelines for the Euthanasia of Animals: 2013 Edition. http://www.avma.org/issues/animal_welfare/euthanasia.pdf.
3. Burns R, McMahan B. Euthanasia methods for ectothermic vertebrates. *In:* Bonagura JD, ed. *Continuing veterinary therapy XII*. Philadelphia: WB Saunders Co. 1995:1379-1381.
4. The Guide for the Care and Use of Laboratory Animals. 2011. National Academy Press, Washington, D.C.
5. Noga, E.J. 2010. Fish Diseases: Diagnosis and Treatment 2nd Ed. Wiley-Blackwell., Ames, Iowa.
6. 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.
7. NIH Guidelines for use of zebrafish: http://oacu.od.nih.gov/ARAC/documents/Zebrafish.pdf
8. University of Washington Environmental Health and Safety - http://www.ehs.washington.edu/