I. **Purpose:** The Brown University IACUC has adopted the following guideline to help research investigators with appropriate preparation, storage and use of tricaine methanesulfonate (MS-222, Tricaine-S). It is the responsibility of the Principal Investigator (PI) to institute adequate inventory and laboratory management procedures to ensure that tricaine methanesulfonate is properly prepared, identified, and stored.

II. **Introduction:**

The use of tricaine methanesulfonate is a common agent used for both fish and amphibious species (i.e. frogs) for temporary immobilization, anesthesia and euthanasia. Due to the acidic nature of tricaine methanesulfonate, the solution must be prepared and buffered appropriately before use in any live, vertebrate aquatic species. Additionally, the Guide for the Care and Use of Laboratory Animals, 8th Edition states (pg. 31), "The use of pharmaceutical-grade chemicals and other substances ensures that toxic or unwanted side effects are not introduced into studies conducted with experimental animals. They should therefore be used, when available, for all animal-related procedures." Thus, the pharmaceutical grade Tricaine-S (Western Chemical) is currently the only FDA-approved version of tricaine methanesulfonate, thus scientific justification in the IACUC protocol must be made if an alternative source (i.e. Sigma) is to be used.

III. **Safe Practices for Working with Tricaine Methanesulfonate (Tricaine-S):**

*Tricane methanesulfonate is not regarded as a toxic chemical by OSHA classification, but it is considered a skin, eye and respiratory irritant, thus appropriate safety precautions should be used.*

- Wear protective clothing, disposable nitrile gloves and safety glasses while handling the Tricaine-S powder.
- Work inside of a fume hood to prepare a concentrated stock solution and dilute the stock solution further as required
- Wear gloves and a utensil to stir the power until dissolved
- Wear gloves to handle animals exposed to Tricaine-S

IV. **Preparation and Storage:**

- **Tricaine-S powder:** can be stored at room temperature. Discard when expiration date on bottle has been reached.
- A 10g/L stock solution can be made and discarded after 30 days, or before the expiration date of the powder from the parent bottle, whichever comes first if it is protected from light (amber glass bottle, wrapped in tin foil) and stored at 4°C. If any degradation is seen (i.e. brown discoloration of solution) the stock must be discarded and should not be used.
All working solutions must be buffered to a pH of 7.0-7.5 by the addition of sodium bicarbonate (NaHCO₃). The pH of the solution must be checked prior to use with either a pH meter or pH paper to ensure neutrality.

Working solutions should not be re-used nor should they be stored for future use. Tricaine-S is light sensitive and quickly is rendered unstable, thus unfit for use.

Note: Disposal must be consistent with established laws and regulations. Tricaine-S should be handled as hazardous waste and disposal coordinated through Brown's Environmental Health and Safety Hazardous Waste Disposal system.

V. Anesthesia
   a. Zebrafish
      i. Tranquilization (non-surgical anesthesia): 20-30 mg/L of Tricaine-S buffered to pH of 7.0 - 7.5.
      ii. Surgical anesthesia: 50-100 mg/L of Tricaine-S buffered to a pH of 7.0 - 7.5.
      iii. Fish should be immersed in solution with a surgical plane of anesthesia reached in approximately 2-3 minutes. The fish can then be removed and anesthesia maintained by dripping solution onto the gills or returning the fish to the anesthetic tank. The fish can be recovered after the procedure by placing it into a recovery tank or by flushing the gills with water.
      iv. Modifications to this Guideline may be needed based on specific fish species, in coordination with the Brown veterinarians.

   b. Frogs
      i. Surgical anesthesia (adult frogs): 1-2 g/L of Tricaine-S buffered to a pH of 7.0-7.5.
      ii. Surgical anesthesia (tadpoles): 0.2-0.5 g/L of Tricaine-S buffered to a pH of 7.0-7.5.
      iii. The tadpole, froglet or frog is immersed in solution with a surgical plane of anesthesia reached in 10-15 minutes. The frog then can be removed and anesthesia maintained by dripping solution onto the skin or by exposure to solution in a petri dish. The frog can be recovered after the procedure by rinsing it in clean, dechlorinated water and placing it into a recovery tank.

VI. Euthanasia

There appears to be substantial species variability in response to MS 222, with some species requiring higher doses or secondary measures to ensure death; please consult with the veterinarians during protocol development for specifics.

   a. Fish
      i. Concentrations >400 mg/L of buffered Tricaine-S constitute an overdose for most fish. Fishes (>7 days post fertilization) should remain in the concentrated solution for a minimum of 10 minutes following cessation of operculum movements. A secondary method of euthanasia is recommended, such as decapitation or rapid freezing in liquid nitrogen.

   b. Frogs
      i. Concentrations of 5-10 g/L of buffered Tricaine-S constitute an overdose for most frog species. Immersion for a minimum of 30 minutes is suggested, however, immersion for as long as 1 hour may be required. Immersion must be followed by an adjunctive method of euthanasia, such as double pithing.

VII. References