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 MS 222 is properly prepared, identified, and stored.

II. **Introduction:**
MS 222 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 MS 222, 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." Pharmaceutical grade MS 222 (Syndel) is currently the only FDA-approved version of MS 222, thus scientific justification is required to be included in the IACUC protocol if a non-pharmaceutic grade source (i.e. Sigma) is to be used.

III. **Safe Practices for Working with Tricaine Methanesulfonate:**
*Tricane methanesulfonate is 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 MS 222 powder.
- Carefully weigh the solid form of MS 222 to minimize aerosolization.
- Work inside of a fume hood to prepare a concentrated stock solution and dilute the stock solution further as required
- Wear personal protective equipment (lab coat or Tyvek, gloves, impact safety glasses) during administration into water and when handling animals exposed to MS 222.

IV. **Preparation and Storage:**
- **MS 222 powder:** store 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. It must be 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 solution must be discarded and should not be used.
- MS 222 must be prepared in water similar to the living condition of the animal. The water should have adequate levels of dissolved oxygen and appropriate pH, temperature, alkalinity, hardness and salinity for the subject. Preferably, water from the water system where the animal is housed should 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. MS 222 is light sensitive and quickly is rendered unstable, thus unfit for use.

V. Disposal
Disposal of all stock, solutions, and products arising from the use of MS 222 must conform to University hazardous waste procedures. MS 222 in used form is not defined as a hazardous waste and may be disposed of via drain disposal if it complies with the Drain Disposal Policy. For questions please contact EHS for guidance.

VI. Anesthesia
1. Zebrafish
   i. *Tranquilization (non-surgical anesthesia):* 20-30 mg/L of MS 222 buffered to pH of 7.0 - 7.5
   ii. *Surgical anesthesia:* 50-100 mg/L of MS 222 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.

2. Frogs
   i. *Surgical anesthesia (adult frogs):* 1-2 g/L of MS 222 buffered to a pH of 7.0-7.5
   ii. *Surgical anesthesia (tadpoles):* 0.2-0.5 g/L of MS 222 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.

VII. 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.*

1. Fish
   i. Concentrations >400 mg/L of buffered MS 222 constitute an overdose for most fish. Adult fishes (>7 days post fertilization) should remain in the concentrated solution for a minimum of 30 minutes following loss of rhythmic operculum movements. Larvae 4-7 days post fertilization should remain in the concentrated solution for a minimum of 20 minutes following cessation of operculum movement. A secondary method of euthanasia is recommended, such as decapitation, pithing, or rapid freezing in liquid nitrogen.
   ii. MS-222 has been shown to be an unreliable euthanasia method for embryos <3 days post fertilization; to ensure embryonic lethality, embryonic exposure to MS-222 should be followed by another agent such as diluted sodium or calcium hypochlorite solution.

2. Frogs
   i. Concentrations of 5-10 g/L of buffered MS 222 constitute an overdose for most frog species. Immersion for a minimum for at least one hour is required to reliably cause euthanasia at these concentrations and should still be followed by a secondary method.
Immersion for a minimum of 20 minutes is permitted but must be followed by an adjunctive method of euthanasia, such as double pithing.³

VIII. References