

Guidelines for the Preparation and Use of MS-222 (Tricaine Methanesulfonate) for Anesthesia and Euthanasia in the African Clawed Frog (*Xenopus laevis*)

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Background

MS-222, or Tricaine Methanesulfonate, is the primary agent of anesthesia and euthanasia for fish and amphibians^{1,2}. For the euthanasia of species of *Xenopus*, the American Veterinary Medical Association regards the use of MS-222 as acceptable but recommends that a secondary method of euthanasia be used to confirm death³.

This document provides guidance regarding safety for personnel preparing solutions of MS-222, appropriate dosages for *Xenopus laevis*, and disposal procedures for MS-222 solutions. All use of MS-222 for animal anesthesia or euthanasia must be approved by the St. Cloud State University IACUC. All activities described herein must be performed by the Principal Investigator (PI) or co-PI(s) named on the IACUC-approved animal use protocol that requires their use. In the absence of these individuals, only the IACUC attending veterinarian or the manager of the animal facility from which the frogs originated may perform these activities.

Safety Precautions

The PI named on an IACUC-approved animal use protocol that requires use of MS-222 is responsible for ensuring the safe use of this compound by personnel and students who engage in any animal use activity involving frogs exposed to MS-222. Personnel who use MS-222 should be familiar with the Safety Data Sheet (SDS) for this compound, which is included as an addendum to this document. MS-222 is a skin, eye, and respiratory irritant.

Solutions of MS-222 should be prepared in a fume hood to minimize risk of inhalation of its powdered form. Examination gloves must be worn at all times when handling powdered MS-222, aqueous solutions of the compound at any concentration, or animals that have been exposed to MS-222. Balances, spatulas, beakers or other laboratory devices and materials used to prepare a solution of MS-222 must be cleaned thoroughly and immediately after use by the individual(s) preparing the solution.

Preparation and Use of MS-222 Solutions

Only ultrapure deionized water may be used for preparation of MS-222 solutions. Deionized water is available in the Biology stockroom (WSB 281) or from any of the deionized water faucets located throughout the ISELF building (e.g., ISELF 320-330 Integrated Research Suite).

Solutions of MS-222 **MUST** be prepared fresh on the day on which they are to be used for anesthesia or euthanasia. NO EXCEPTIONS. Since MS-222 can acidify the water in which it is solubilized, all MS-222 solutions **MUST** be buffered to a pH of 7.0 with sodium bicarbonate before use. Prepare **ONLY** as much MS-222 solution as is needed on a single day.

For Surgical Anesthesia:

- Dispense 1 liter of ultrapure deionized water into a clean beaker, flask or other suitable container.

- Dispense 2 grams of powdered MS-222 into a small disposable weigh boat on an electronic balance. Make certain that the balance is tared (i.e., zeroed with a clean, empty weigh boat situated atop the weighing pan) before dispensing powdered MS-222 into the weigh boat.
- Transfer the 2 grams of powdered MS-222 from the weigh boat into 1 liter of deionized water and stir until solubilized completely. MS-222 solubilized in this manner will form a clear solution.
- Measure the pH of the solution using an electronic pH meter. Make certain to calibrate the pH meter per the unit's instructions prior to use.
- Add sodium bicarbonate to the solution in 1- to 2-gram increments and measure the pH of the solution using an electronic pH meter following the addition of each increment. Make certain that the sodium bicarbonate has dissolved completely before each measurement of the pH. The solution should remain clear as the sodium bicarbonate is solubilized.
- When the MS-222 solution reaches a pH of 7.0, the solution may be used for anesthesia.

Note: For animal use activities that require more than 1 liter of anesthetic MS-222 solution, multiply the values for *mass and volume* in the formula above by the desired number of liters of MS-222 solution. Regardless of the final volume of MS-222 solution desired, the pH must be 7.0 prior to use. No exceptions.

- Transfer the freshly prepared MS-222 solution into an approximately rectangular container with sides that are at least 10 inches in height to prevent escape of frogs. The container must be clean and sanitary prior to use and it must be labelled appropriately with the concentration of MS-222 indicated in grams/liter. A standard 10-gallon aquarium containing between 1.5 and 2 liters of MS-222 solution works very well for this purpose – see images below.

IMPORTANT! The depth of the solution *must* be sufficient to cover as much of the body of each frog as possible, *while keeping the head and nares above the surface of the solution*. This measure is intended to prevent accidental drowning during the induction of anesthesia.



Figure 1a. Side view of a standard 10-gallon aquarium containing 2 liters of water and 12 frogs.



Figure 1b. Top view of a standard 10-gallon aquarium containing 2 liters of water and 12 frogs.

- **ALL animals placed in an MS-222 solution for the induction of surgical anesthesia must remain in the solution for *no less than 20 minutes!* NO EXCEPTIONS.**

Dosing adult *Xenopus laevis* in this manner should provide at least 60 minutes of surgical anesthesia⁴.

SURGICAL PROCEDURES DESCRIBED IN AN ANIMAL USE PROTOCOL INVOLVING ADULT *XENOPUS LAEVIS* MUST TAKE INTO ACCOUNT THIS EXPECTED DURATION OF SURGICAL ANESTHESIA AND MUST LIMIT THE LENGTH OF ANIMAL USE ACTIVITIES ACCORDINGLY.

For Euthanasia

Frogs that are anesthetized by the procedure described above for surgical anesthesia may be euthanized by decapitation. Euthanasia by this method, however, **MUST** be performed within 60 minutes following removal of a frog from the anesthetic MS-222 solution (i.e., 2g MS-222/L). This ensures that the animal is in a state of surgical anesthesia at the time of decapitation.

For terminal surgical procedures, each animal used must be decapitated *immediately* upon completion of its use in a procedure in order to confirm death before the animal's carcass may be discarded.

For animal use activities in which MS-222 is used solely as an agent of euthanasia, the MS-222 solution must be prepared as follows:

- Dispense 1 liter of ultrapure deionized water into a clean beaker, flask or other container.
- Dispense 5 grams of powdered MS-222 into a small disposable weigh boat on an electronic balance. Make certain that the balance is tared (i.e., zeroed with a clean, empty weigh boat situated atop the weighing pan) before dispensing powdered MS-222 into the weigh boat.
- Transfer the 5 grams of powdered MS-222 from the weight boat into 1 liter of deionized water and stir until solubilized completely. MS-222 solubilized in this manner will form a clear solution.
- Measure the pH of the solution using an electronic pH meter. Make certain to calibrate the pH meter per the unit's instructions prior to use.

- Add sodium bicarbonate to the solution in 1- to 2-gram increments and measure the pH of the solution using an electronic pH meter following the addition of each increment. Make certain that the sodium bicarbonate has dissolved completely before measuring the pH. The solution should remain clear as the sodium bicarbonate is solubilized.
- When the MS-222 solution reaches a pH of 7.0, the solution may be used for euthanasia.

Note: If the number of frogs to be euthanized simultaneously requires more than 1 liter of MS-222 solution, multiply the values for *mass and volume* in the formula above by the desired number of liters of MS-222 solution. Regardless of the final volume of MS-222 solution desired, the pH must be 7.0 prior to use. No exceptions.

- Transfer the freshly prepared MS-222 solution into an appropriately sized container that will permit complete immersion of all frogs placed into the MS-222 solution. The container must be clean and sanitary prior to use and it must be labelled appropriately with the concentration of MS-222 indicated in grams/liter. A standard 10-gallon aquarium containing a minimum of 3 liters of MS-222 solution works very well for this purpose.

The depth of the solution **must** be sufficient to cover all animals completely at all times. The solution should permit all animals to be immersed to a depth of no less 1 inch below the surface of the solution.

Animals placed in an MS-222 solution for euthanasia must remain in the solution for no less than 60 minutes. NO EXCEPTIONS. This is the minimum amount of time required to ensure death of all frogs immersed in a solution of MS-222 at a concentration of 5g/liter⁵.

In the event that frogs must be immersed in a solution of MS-222 for less than 60 minutes, they must be decapitated in order to confirm death. The frogs must remain in the MS-222 solution, however, for a minimum of 20 minutes before decapitation may be performed.

Disposal of MS-222 Solution

All solutions of MS-222 must be discarded on the day on which they are prepared. A solution of MS-222 may not be used on more than one day.

A solution of MS-222 prepared at either of the concentrations described above may be discarded via a sanitary sewer. At a sink, open both valves on the faucet (maximum flow of tap water) and pour the MS-222 solution slowly into the sink to allow the solution to be flushed with copious water.

References

1. Downes, U. 1995. Tricaine methanesulfonate in amphibians: a review. *Bulletin of the Association of Reptilian and Amphibian Veterinarians* 5: 11-16.
2. Wright, KM. 1996. Amphibian husbandry and medicine, pp. 436-58. In: Mader, DR. *Reptile medicine and surgery*. Philadelphia (PA): WB Saunders.
3. Leary, S., et al. 2020. *AVMA Guidelines for the Euthanasia of Animals: 2020 edition*. American Veterinary Medical Association, Schaumburg, Ill, USA, 2020.
4. Lalonde-Robert, V., F. Beaudry, and P. Vaschon. 2012. Pharmacologic parameters of MS222 and physiologic changes in frogs (*Xenopus laevis*) after immersion at anesthetic doses. *Journal of the American Association for Laboratory Animal Science* 51: 464-68.
5. Torreilles, S., D. McClure, and S. Green. 2009. Evaluation and refinement of euthanasia methods for *Xenopus laevis*. *Journal of the American Association Laboratory Animal Science* 48: 512–16.