SOUTHERN ILLINOIS UNIVERSITY CARBONDALE
Institutional Animal Care & Use Committee

Standard Operating Procedure (SOP) for
Tricaine Methanesulfonate (MS-222) Safety, Preparation, and Use

Purpose:
Tricaine methanesulfonate (MS-222) is a commonly used as an anesthetic and AVMA approved euthanasia agent for fish, amphibians, and reptiles. MS-222 does not meet the OSHA classification as a toxic chemical but is considered to be an irritant to the eyes, respiratory system, and skin. This document provides guidance on the safe use of MS-222.

Definitions:
Pharmaceutical grade: a drug approved by the Food and Drug Administration (FDA) with an established level of purity, defined bioavailability, defined routes, and defined half-life elimination. The FDA-approved drugs for veterinary use are listed in the Green Book and drugs approved for human use are listed in the Orange Book.

USP/NF: the United States Pharmacopoeia (USP) National Formulary (NF) is the official public standards-setting authority for prescription and non-prescription drugs in the U.S. These drugs typically have the letters USP/NF after the drug listing. If these drugs are approved by the FDA and listed in the Green Book or Orange Book, they are pharmaceutical grade. However, if they are NOT listed in the Green Book or Orange Book, then they are NOT pharmaceutical grade. Thus, a USP/NF grade chemical may or may not be pharmaceutical grade.

Non-pharmaceutical grade: a chemical not formulated for use in animals or humans. These may be identified as USP/NF grade but not listed in the Green or Orange Books; they also may be listed as analytical grade, reagent grade, USP purity grade, or analytical standards.

Personnel Safety:
Avoid breathing dust/fume/gas/mist/vapors spray. If possible, work inside of a fume hood to prepare a concentrated stock solution by mixing the appropriate amount of MS-222 powder in a small volume of water. If you must work outside of a chemical fume hood in a laboratory, carefully weigh the solid in a location that has minimal air disturbance.

If you must prepare MS-222 in the field, don appropriate PPE (protective clothing, gloves,

safety glasses, mask) and work in well-ventilated area. Avoid working with the powder in a location that could cause the powder to blow into your breathing zone or unprotected skin. It is good practice to pre-weigh amounts of MS-222 into vials that can then be transferred into the desired volume of water at the desired field location.

Use of a particulate respirator (N-95) is not required for typical applications in research labs or in the field. As long as staff are careful during the weighing of the solid, there is minimal potential for exposure to the pure MS-222. Use of an N-95 respirator will help prevent inhalation in the event of accidental aerosolization, and is encouraged for those seeking additional protection, or who are new to carrying out the procedure.

Contact SIU's Center for Environmental Health and Safety (CEHS) to assist with risk assessment if non-typical applications (e.g., use of large quantities, long exposure duration) are being considered.

Exposure Response:

- If on skin, wash with plenty of water.
- If inhaled, remove person to fresh air and keep comfortable for breathing.
- If in eyes, rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
- If ingested, do not induce vomiting. Rinse mouth with water and seek medical attention.
- Call a poison center/doctor if you feel unwell.
- If skin or eye irritation occurs, get medical attention.
- Take off contaminated clothing and wash before reuse.

Use of MS-222:

MS-222 is a benzoic acid derivative and, in water of low alkalinity (<50 mg/L as $CaCO_3$), the solution should 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.

The stock solution should be protected from light and refrigerated or frozen if possible. The solution should be replaced within 10 days and any time a brown color is observed.

Potency is increased in warm water and decreased in cold water. Immersion of fish in solutions of MS-222 for 10 minutes following loss of rhythmic opercular movement is sufficient for euthanasia of most fish. Juvenile salmon or small cyprinids species may experience loss of opercular movement within 5 minutes at a water concentration of 300 mg/L of sodium bicarbonate buffered MS-222 at a temperature range of 10-25°C. Euthanasia by MS-222, including methods of confirming and ensuring death, must be described in the accompanying animal use protocol.

In the United States, there is a 21-day withdrawal time for MS-222; therefore, it is not appropriate for euthanasia of animals intended for consumption. If using MS-222 for euthanasia, a secondary method should be used to ensure death.

Disposal:
It is important to only generate the amount of MS-222 solution you need to perform the protocol. When using MS-222 on campus, all unused or expired MS-222 powder and aqueous solutions must be properly disposal through CEHS.

While working off campus or in the field, MS-222 waste solution may be disposed of by flushing the solution down the drain to a sanitary sewer with excess water **IF** the dilution is no more than 1% MS-222 and the pH is between 6-10. Solid MS-222 (powder or tablets) should be returned to campus for disposal through the CEHS.

Carcasses exposed to MS-222 should be placed in a red biohazard bag and frozen until they can be picked up by CEHS. CEHS requires that a biological waste pickup form be completed and submitted so they can schedule the pick-up.

Protocol Considerations:
Use of MS-222 should be described in the IACUC approved protocol. Please note that once MS-222 is diluted, **it is no longer pharmaceutical grade.**

Per IACUC Policy #570, *Use of Pharmaceutical and Non-pharmaceutical Grade Drugs*, pharmaceutical grade substances must be used, when available, for all vertebrate and cephalopod animal procedures, even if the final dilution is not pharmaceutical grade. If a pharmaceutical grade powder is unavailable, or cannot be used in a specific study, the IACUC will review the justification described in the protocol.

Please pay particular attention to the Drugs and Compounds section of the protocol.

- Part H1 – Ensure that MS-222 is listed and state that it will be diluted and buffered.
- Part H2 – Indicate that not all chemicals and substances used will be pharmaceutical grade using the appropriate check box.
- Part H2 - In the appropriate bullet point, describe methods used to formulate the dose given to animals, ensuring the sterility of the non-pharmaceutical grade compound.
- Part H2 - In the appropriate bullet point, include justification for using a non-pharmaceutical grade compound (e.g., it must be diluted).

References:

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