

 BROWN	Institutional Animal Care and Use Committee (IACUC): Policy on Preparation, Storage, Handling and Use of Tricaine Methanesulfonate	POL 10.10.17
		Effective Date: November 23, 2020

1.0 Policy Purpose

The purpose of this policy is to provide guidance to Principal Investigators (PIs) regarding appropriate preparation, storage, and use of tricaine methanesulfonate (otherwise referred to as Tricaine-S or MS-222).

2.0 To Whom the Policy Applies

This policy applies to all individuals involved in live vertebrate research at Brown University under an IACUC-approved animal use protocol.

3.0 Policy Statement

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. It is the responsibility of the PI to institute adequate inventory and laboratory management procedures to ensure that tricaine methanesulfonate is properly prepared, identified, and stored.

The Guide for the Care and Use of Laboratory Animals, 8th Edition states: *"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"*(p. 31). Pharmaceutical grade tricaine methanesulfonate from Syndel is currently the only FDA-approved version, thus scientific justification is required in any animal use protocol for consideration by the IACUC if a non-pharmaceutic grade source (*i.e.*, Sigma) is proposed for use.

3.1 Safe Practices for Working with Tricaine Methanesulfonate

Tricaine methanesulfonate is a skin, eye, and respiratory irritant; thus, appropriate safety precautions must be taken with its use. The following precautions must be taken by personnel who work with tricaine methanesulfonate:

- Personnel must wear protective clothing, disposable nitrile gloves, and safety glasses while handling the powder form.

- Personnel must carefully weigh the solid form of tricaine methanesulfonate to minimize aerosolization.
- Personnel must work inside of the fume hood to prepare a concentrated stock solution and dilute the stock solution further as required.
- Personnel must wear personal protective equipment (*i.e.*, lab coat or Tyvek, gloves, and impact safety glasses) during administration of tricaine methanesulfonate into water and when handling animals exposed to tricaine methanesulfonate.

3.2 Preparation and Storage

The following guidelines must be followed for preparation and storage of Tricaine methanesulfonate:

- Tricaine methanesulfonate in powder form must be stored at room temperature and discarded when the 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.
- Tricaine methanesulfonate must be protected from light (*e.g.*, in an amber glass bottle, wrapped in tin foil) and stored at four degrees Celsius. If any degradation is seen (*e.g.*, brown discoloration of solution) the stock solution must be discarded and must not be used.
- Tricaine methanesulfonate must be prepared in water similar to the living condition of the animal. The water must have adequate levels of dissolved oxygen and appropriate pH, temperature, alkalinity, hardness, and salinity for the animal. It is preferable that water from the water system where the animal is housed 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 must not be re-used or stored for future use. Tricaine methanesulfonate is light sensitive and quickly is rendered unstable, and thus unfit for use.

3.3 Disposal

Disposal of all stock, solutions, and products arising from the use of tricaine methanesulfonate must conform to University hazardous waste procedures. Tricaine methanesulfonate in its used form is not defined as a hazardous waste and may be disposed of via drain disposal if it complies with the Environmental Health and Safety's [Drain Disposal Policy](#).

3.4 Tricaine Methanesulfonate as Anesthesia

The following guidelines detail the way in which researchers must use tricaine methanesulfonate as an anesthetic in zebrafish and frogs.

3.4.1 Zebrafish

- Tranquilization (non-surgical anesthesia): 20-30 mg/L of tricaine methanesulfonate buffered to pH of 7.0 - 7.5.

- Surgical anesthesia: 50-100 mg/L of tricaine methanesulfonate buffered to a pH of 7.0 - 7.5.
- Zebrafish must be immersed in solution with a surgical plane of anesthesia reached in approximately two to three 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.
- Modifications to these guidelines may be necessary based on specific fish species. Such modifications must be made in coordination with a Center for Animal Resources and Education (CARE) veterinarian.

3.4.2 Frogs

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

3.5 Euthanasia

There is substantial species variability in response to tricaine methanesulfonate, with some species requiring higher doses or secondary measures to ensure death. As such, PIs must consult with a CARE veterinarian during IACUC protocol development to determine appropriate dosing for euthanasia. The following guidelines detail the way in which researchers must use tricaine methanesulfonate as a euthanasia agent in fish and frogs, unless otherwise instructed by a CARE veterinarian.

3.5.1 Fish

- Concentrations >400 mg/L of buffered tricaine methanesulfonate constitute an overdose for most fish. Adult fishes (>14 days post fertilization (dpf)) must remain in the concentrated solution for a minimum of 30 minutes following loss of rhythmic operculum movements. Larvae 4-7 dpf must 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.
- Tricaine methanesulfonate has been shown to be an unreliable euthanasia method for zebrafish eggs, embryos, or larvae <14 dpf as a single-step method. Thus, zebrafish <14 dpf should remain in a buffered tricaine methanesulfonate >400 mg/L solution for a minimum of 30 minutes immediately followed by a **required** secondary method of euthanasia, such as

decapitation, pithing, rapid freezing in liquid nitrogen or diluted sodium, or calcium hypochlorite solution for larvae <7 dpf.

3.5.2 Frogs

- Concentrations of 5-10 g/L of buffered tricaine methanesulfonate 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 must be followed by a secondary method. Immersion for a minimum of 20 minutes is permitted but only if it is followed by an adjunctive method of euthanasia, such as double pithing.

4.0 Definitions: N/A

5.0 Responsibilities

All individuals to whom this policy applies are responsible for becoming familiar with and following this policy. University supervisors are responsible for promoting the understanding of this policy and for taking appropriate steps to help ensure compliance with it.

6.0 Consequences for Violating this Policy

Violation of this policy may be considered a serious event of noncompliance that is reportable to the IACUC, funding and accrediting agencies, as well as other regulatory agencies. Violation of this policy is a serious matter that may adversely affect both the ability to perform animal work and acquire funding sources.

Failure to comply with this and related policies is subject to disciplinary action, up to and including suspension without pay, or termination of employment or association with the University, in accordance with applicable (*e.g.*, staff, faculty, student) disciplinary procedures.

7.0 Related Information

Brown University is a community in which employees are encouraged to share workplace concerns with University leadership. Additionally, [Brown's Anonymous Reporting Hotline](#) allows anonymous and confidential reporting on matters of concern online or by phone (877-318-9184).

The following information complements and supplements this document. The information is intended to help explain this policy and is not an all-inclusive list of policies, procedures, laws and requirements.

7.1 Related Policies:

- [Brown University EHS Drain Disposal Policy](#)

7.2 Related Procedures: N/A

7.3 Related Forms: N/A

7.4 Frequently Asked Questions (FAQs): N/A

7.5 References:

- American Veterinary Medical Association. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition.
- Collymore C, Banks KE, Turner PV. Lidocaine hydrochloride compared with MS222 for the euthanasia of zebrafish (*Danio rerio*). *J Am Assoc Lab Anim Sci*. 2016;55:816–820.
- Collymore C, Tolwani A, Lieggi C, Rasmussen S. Efficacy and safety of 5 anesthetics in adult zebrafish (*Danio rerio*). *J Am Assoc Lab Anim Sci*. 2014;53(2):198–203.
- Flecknell, P. *Laboratory Animal Anesthesia*. Elsevier, 2009. Print edition.
- Green, S., *The Laboratory Xenopus sp.* CRC Press, 2010. Print edition.
- Harper, C., and Lawrence, C. *The Laboratory Zebrafish*. CRC Press, 2011. Print edition.
- Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals*, 8th Edition. National Academies Press, 2011.
- Torreilles SL, McClure DE, Green SL. Evaluation and refinement of euthanasia methods for *Xenopus laevis*. *J Am Assoc Lab Anim Sci* 2009;48:512–516.
- Vargas RA. Anesthesiology, anesthetics and zebrafish (*Danio Rerio*). An animal model to perform basic biomedical research. *EC Anaesthesia*. 2018;4.6:202-213.

8.0 Policy Owner and Contact

8.1 Policy Owners: Vice President for Research

8.2 Policy Approved by: Vice President for Research

8.3 Subject Matter Contact: Brown University Animal Research Protection Program

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9.0 Policy History

9.1 Policy Effective Date: November 4, 2016

9.2 Policy Last Reviewed: November 23, 2020

9.3 Policy Update/Review Summary: This policy is not new; it was converted to the University's new policy template and re-reviewed by the IACUC at its convened meeting on April 3, 2020. Previous version in the old policy format superseded by this version:

- Guidelines for Preparation, Storage, Handling and Use of Tricaine Methanesulfonate (Tricaine-S, MS-222), Date of IACUC Approval: November 4, 2016, Last Updated: February 7, 2020