UC RIVERSIDE | Environmental Health & Safety

Tetrodotoxin

Standard Operating Procedure

All research involving the materials described in this SOP must be documented and approved in a Biological Use Authorization (BUA) prior to use.

RISK GROUP 2

Approved BUA #

BUA Expiration Date

Description

Tetrodotoxin (TTX) is a natural toxin found in some fish such as puffer fish and toad fish. TTX acts as a sodium channel blocker on both central and peripheral nervous systems and causes muscle paralysis. TTX is commonly used in neurological research.

Tetrodotoxin poisoning may be rapid (10-45 minutes) or delayed onset (3-6 hours). Effects of exposure include numbness and tingling (paresthesia) of lips and tongue, facial and extremity paresthesia, headache, profuse sweating, nausea, difficulty breathing or shortness of breath.

Tetrodotoxin is a Select Agent toxin. No laboratory/principal investigator may possess more than 500mg of TTX without prior approval from the UCR High Containment Lab Director and Federal Select Agent Program (FSAP). Holding more than 500mg of Tetrodotoxin without FSAP approval is a federal offense punishable by up to $500,000 and 5 years imprisonment.

Personal Protective Equipment

- Closed-toe shoes; long pants or equivalent
- Long-sleeved lab coat
- Safety glasses or goggles when a splash risk is present
- Non-permeable, nitrile gloves

- Static-free nitrile gloves required when handling dry toxin (powder)
- N-95 respirator may be required for handling powder. N95 users must be fit tested and complete a medical evaluation. Contact ehsbiosafety@ucr.edu for more information.

Engineering Controls

A certified fume hood or Biosafety Cabinet (BSC) is required for handling powder or when performing procedures that can produce aerosols.

Procedures

PREPARATION AND USE.

Tetrodotoxin (TTX) should be purchased from suppliers in the smallest amount feasible. If package is damaged, contact the vendor immediately and decontaminate the package by donning appropriate PPE and placing the package in fume hood or BSC. Saturate with freshly-made 20% bleach. Dispose solid waste into red biohazard bag. Wait at least 60 minutes then dispose of the package into biohazardous waste.

Routine operations with toxin powder and solutions are conducted under **BSL-2** conditions:

- Design experiments to minimize or eliminate work with dry toxin
- Restrict access to area where TTX is handled
- Always place an absorbent pad on the work surface before handling toxin
- Procedures involving dry toxin are restricted to fume hood or BSC
- Aerosol-generating procedures are restricted to fume hood or BSC
- Vacuum lines must be protected by a HEPA filter
- Substitute plastic for glass whenever possible

STORAGE.

TTX stocks should be stored in clearly marked secondary container labeled with the name of the toxin. **All Tetrodotoxin must be kept secured within a locked cabinet or freezer and inventoried by the laboratory.** A bound notebook containing the log of TTX usage should be kept in the lab. At all times the PI must be able to account for all TTX purchased/obtained by the laboratory. The secured TTX should be in a secondary container clearly labeled with contact information for trained, responsible laboratory staff.

TRANSPORT.

Tetrodotoxin should be transported in a leak-proof primary container labeled with the hazard name then placed into a leak-proof, rigid, non-breakable secondary container clearly labeled with the biohazard symbol as well as PI name and contact information. If TTX is in liquid

solution, the secondary container should also contain enough absorbent material to prevent seepage of any spill. Transport of TTX to vivaria locations must be approved by the IACUC and be reflected in the PI's Animal User Protocol (AUP).

Decontamination and Waste

- All work surfaces should be decontaminated with freshly-made 10% bleach.
- Liquid TTX waste (even dilute amounts) containing no other hazardous chemicals must be decontaminated with fresh bleach to a final concentration of 10% bleach. Allow at least 30 minutes contact time. Afterwards, the decontaminated liquid solution can be disposed down the drain with copious amounts of water.
- Liquid TTX waste containing other hazardous chemicals should be disposed of as hazardous chemical waste.
- Gloves and other contaminated solids should be disposed of as biohazardous waste.
- Contaminated glassware must be soaked with 10-20% bleach for at least 30 minutes prior to washing.
- Contaminated sharps should be placed into a chemical sharps container. Do not recap needles.

Spills

POWDER SPILL.

Avoid generating dust. With gloved hands, remove any contaminated clothing and put clothing in red biohazard bag. Notify other workers in area of the spill and prevent traffic in the area. If spill is not contained inside a fume hood or ducted biosafety cabinet, all personnel should immediately leave the area for at least 30 minutes to allow any toxins to be dispersed by building HVAC. Post a signage at the entrance to warn personnel not to enter. Monitor personnel for signs of exposure.

After 30 minutes, clean up the spill. Follow the liquid spill procedure below and place paper towels on surfaces contaminated with dust. Be careful not to generate powder dust when covering the area with paper towels.

LIQUID SPILL.

If the spill is on the floor, use shoe covers. Put on fresh gloves and cover spill area with paper towels. Pour freshly made 10% bleach over paper towels from edge of spill to center; be careful not to splash bleach. All objects in spill area should be decontaminated with 10% bleach. Allow 30 minutes of contact time for the bleach.

If there are any sharps, including broken glass, use forceps or tongs to pick up and place into sharps container. Place bleach-soaked paper-towels into biohazardous waste bag and wipe area with bleach and paper towels again. Mop if needed. Remove glove and foot covers and place into red biohazard bag.

All spills must be reported to EH&S and lab supervisor within 24 hours.

EH&S contact information

Phone: (951) 827-5528

Email: biosafety@ucr.edu

Website: https://ehs.ucr.edu/ ('Report Incident' link at top of page)

First Aid & Emergencies

SKIN.

Wash with soap and water for 15 minutes. Carefully remove any contaminated clothing and dispose of as biohazardous waste. Report incident to supervisor and EH&S. Seek medical attention if needed.

NEEDLESTICK / SHARPS INJURY.

Flush wound with soap and water. Immediately seek medical attention.

INGESTION.

Do not induce vomiting. Immediately seek medical attention. Report incident to supervisor and EH&S.

MUCOUS MEMBRANE.

Flush at eyewash for at least 15 minutes. Seek immediate medical attention. Report incident to supervisor and EH&S.

All exposures must be reported to EH&S and lab supervisor within 24 hours.

EH&S contact information

Phone: (951) 827-5528

Email: biosafety@ucr.edu

Website: https://ehs.ucr.edu/ (Online reporting link at top of page)

References

- CDC NIOSH – Tetrodotoxin

  https://www.cdc.gov/niosh/ershdb/emergencyresponsecard_29750019.html

- Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition – Appendix I—Guidelines for Work with Toxins of Biological Origin

  https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf

UC RIVERSIDE | Environmental Health & Safety

Acknowledgement

By signing below I acknowledge that I have read, understand, and agree to abide by the procedures and practices described in this document.

_____ _____

Principal Investigator **Date**

Name	Signature	Date

UC RIVERSIDE | Environmental Health & Safety

Dry Toxin Usage Log

Tetrodotoxin is a select agent and is exempt from select agent restrictions only if the total amount of toxin owned by a single investigator is under ***500 mg.***

Product: _____

Starting Amount: _____

Purchase Date: _____

Purchase Date: _____

Date	Amount Remaining	Amount Used	User	Reason for Use