

SOP: Use of Toxins of Biological Origin

Toxin: Tetrodotoxin (TTX)

**Labs can only store a total concentration of less than 500mg of TTX at all times.**

An IBC application is required for the use of Select Toxins in any amount. The Select Toxins list can be found here: <https://www.selectagents.gov/SelectAgentsandToxinsList.html>. If the amount of select toxin you are planning to use exceeds the permissible amounts (<https://www.selectagents.gov/PermissibleToxinAmounts.html>), additional requirements apply – please contact Biosafety ([biosafety@uci.edu](mailto:biosafety@uci.edu)) for more information.

**LAB INFORMATION**

Principal Investigator:

Phone:

Email:

Alternate:

Phone:

Email:

IBC Protocol #

IACUC Protocol # (if applicable)

Locations of work:

**SUBSTANCE INFORMATION**

Substances Product Name:

Synonyms:

Form (physical state):

Formula:

Molecular Weight:

CAS Number:

Source (include catalog #):

Purpose of use (in vitro, in vivo):

**APPROVED LOCATIONS**

**Preparation**: *[location of designated certified fume hood and/or biosafety cabinet]*

**Experiments**: *[include animal areas, if applicable]*

The working locations are required to have posted signage (Caution: Toxin Use Area) and have a door placard indicating minimum PPE requirements (fitted lab coat or disposable long-sleeved protective gown, disposable nitrile gloves, eye protection – safety glasses or googles).

**STORAGE AND SECURITY**

Vials of TTX stock solutions and their aliquots must be stored inside a dedicated secondary leak-proof, anti-shatter secondary container labeled with the contents and “Toxin.” **Access to samples is restricted only to authorized users.**

**SAFETY REQUIREMENTS**

* Prior to work, ensure that the fume hood or biosafety cabinet (BSC) has been certified within the year and is working properly. Perform all work within the operationally effective zone of the fume hood or BSC. Check that the room has negative pressure (air flowing into the room).
* Standard BSL2 practices should be employed. No eating, drinking, application of lip balms/makeup or storage of food in the work area. No mouth pipetting. Appropriate PPE must be worn.
* Remove PPE before leaving laboratory areas.
* Wash hands with soap and water after completion of the procedure and before leaving the laboratory.

**Training**

**Only trained and authorized lab members are allowed to handle TTX.**

All personnel possessing, using, transferring, or receiving select toxins must have documented training on the contents of this SOP, the Chemical Hygiene Plan, select toxin-specific laboratory work, the TTX SDS, and have completed the Select Agents course on UCLC. Training is required before initiation of research involving select toxins and annually thereafter. The PI must maintain all records of training.

**Personal Protective Equipment (PPE)**

Use of personal protective equipment (PPE) is required at all times for all procedures including reconstitution, handling of stocks, and performing experiments. Minimum PPE includes: **1)** fitted lab coat or disposable long-sleeved protective gown, **2)** disposable nitrile gloves (double gloves) and **3)** eye protection (safety glasses or googles). Face protection such as a face shield over safety glasses will be worn if splash/spatter is possible. Change gloves and discard in designated chemical waste container immediately if contaminated, torn or punctured.

**Handling**

* Containers of TTX must be handled in a certified BSC or fume hood. Signage (Caution: Toxin Use Area) will be posted on the room door and in areas where TTX is handled. Signage must include minimum PPE requirements.
* To facilitate cleanup and to help contain spills, a plastic-backed absorbent material may be placed on the work surface (without blocking any air grilles).
* Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Containers containing the powder form must never be opened under any circumstances.
* Use blunt-fill needles to reconstitute powdered TTX through the rubber septum. Sharps should be avoided whenever possible. If needles are required, they must never be re-capped, and must be disposed of in a sharps waste container immediately after use. While working with sharps inside the BSC, the sharps container must be kept inside the BSC.
* Centrifugation: Centrifuge tubes should be prepared and sealed/loaded and unloaded in the rotor/buckets in the biosafety cabinet. This includes methods to ensure tubes are properly balanced (unless the balance tube contains no infectious material). At the end of the procedure, rotors and/or buckets must be decontaminated.
* Vortexing must be done in the certified biosafety cabinet or chemical fume hood.

**Transport**

If TTX or any materials treated with TTX are to be transported, a secondary container is required to contain the materials. The secondary container must be leak proof, rigid, and shatter-proof container with a tight-fitting lid and labeled Toxin.

**Decontamination and Disposal:**

* Additional procedures are required for destruction of inventory stocks. See INVENTORY LOG AND DESTRUCTION PROCEDURES section below.
* All waste contaminated or potentially contaminated with TTX should be soaked in 20% bleach solution (~1% sodium hypochlorite) for at least 30 minutes before disposal in a hazardous chemical waste container labeled as “Inactivated Tetrodotoxin (TTX) in sodium hypochlorite.”.
* Work surfaces must be wiped down with 20% bleach (wet contact time 30 minutes) after each procedure or when spills/splashes occur. Metal surfaces must be followed with a generous sterile water wipe down to prevent corrosion.
* Liquid waste (non-stock inventory only) will be treated with 20% solution bleach (1 part bleach to 4 parts waste) for at least 30 minutes and then collected in a liquid chemical waste container labeled as “Inactivated Tetrodotoxin (TTX) in sodium hypochlorite.”
* Solid or liquid waste contaminated with TTX must be discarded and collected as hazardous chemical waste in properly labeled and properly stored containers for downstream incineration.
* Hazardous chemical waste must be ordered for collection within 6 months of the start of waste generation. For chemical waste pick up go online at https://www.ehs.uci.edu or call 949-824-6200 to arrange pick-up.

**INVENTORY LOG AND DESTRUCTION PROCEDURES**

**Inventory Log**. An inventory of use of TTX must be available and kept in the lab once TTX use is active. A spreadsheet with the following information must be available: Date, User Initials, Initial Amount, Remaining Amount. Input information as material is used. An annual inventory check will be scheduled with the lab and conducted by the Biosafety Officer.

**Destruction procedures** (for final destruction of TTX stocks): 1) complete EH&S select toxin destruction form, 2) destruction should be performed inside the certified BSC or fume hood and must be witnessed by EH&S biosafety staff. Contact EH&S at 4-6200 to arrange a destruction meeting.

**SPILL MANAGEMENT**

The lab must have a spill kit, or the components of such readily accessible in the event of a spill. This comprises: an easy-to-read outline of the spill response SOP; gloves, masks, goggles, clean lab gown or lab coat, paper towels to absorb contaminated liquids, disinfectant, tongs or forceps to pick up broken glass, an appropriate hazardous chemical waste container.

Always wear the minimum required PPE when performing spill clean up. Additional PPE (e.g. face shields) may be required depending on the nature of the spill. Wash hands thoroughly after completing any spill cleanup. The lab’s spill kit is located in [insert location].

***Note: Only clean up spills if you have been trained to do so and that you are comfortable cleaning. For assistance with spill cleanup, please contact EH&S (949) 824-6200.***

* In case of accidental **liquid spills inside** of the BSC or fume hood: Do not turn off the BSC or fume hood. Cover spill with absorbent paper towels and apply 20% bleach solution, starting at the perimeter and working towards the center, allowing a 30 minutes contact time to inactivate TTX. Clean the spill area again with bleach. Solid waste will be double bagged and disposed of in a properly labeled hazardous chemical waste container. Wash hands thoroughly after completing any spill cleanup.
* In case of accidental **powder spills inside** of the fume hood or BSC: Do not turn off the BSC or fume hood. Gently cover powder spill with dampened absorbent paper towels to avoid raising dust. Apply 20% bleach solution (1% sodium hypochlorite), starting at the perimeter and working towards the center, allowing a 30-minute contact time to inactivate TTX before clean-up. Clean the spill area again with bleach. The decontaminated spill waste will be double bagged and disposed of in a properly labeled hazardous chemical waste container. Wash hands thoroughly after completing any spill cleanup.
* For **powder or liquid spills outside** of the fume hood or BSC: Inform all personnel to leave the room and restrict access. As soon as possible report the spill by notifying EH&S at 949-824-6200. Also notify PI or lab supervisor. When calling EH&S, be prepared to provide the following information: name and phone number of knowledgeable person that can be contacted; name of chemical, concentration and amount spilled; number of injured, if any; location of spill.

**POST-EXPOSURE MANAGEMENT**

* Refer to the **Exposure Control Plan** for reporting and documentation requirements for injuries, and post-exposure evaluation and follow-up.
* Review and know the contents of the **UCI Injuries and Medical Treatment poster** prior to handling TTX. Call 911 immediately for life-threatening emergencies.
* Report all incidents and injuries **within 24 hours** to EH&S: <https://www.ehs.uci.edu/forms/report-injury/index.php>

TTX can enter the body through ingestion, inhalation, injection, or abraded skin.

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| Symptoms of Tetrodotoxin exposure include:   * Tingling of the tongue and mouth * Vertigo or dizziness * Feelings of doom * Weakness * Nausea and vomiting | Signs of Intoxication include:   * Ascending paralysis * Respiratory paralysis * Convulsions * Salivation * Muscle twitching * Fixed dilated pupils | Treatment:   * No specific antitoxin is available * Supportive care * Anticipation of progressive ascending paralysis, particularly of the respiratory system * Stomach evacuation by the emergency department if ingested |

* **TIME COURSE**: Tetrodotoxin poisoning may either have rapid onset (10 to 45 minutes) or delayed onset (generally within 3 to 6 hours but rarely longer). Death may occur as early as 20 minutes, or as late as 24 hours, after exposure; but it usually occurs within the first 4 to 8 hours. Patient/victims who live through the acute intoxication in the first 24 hours usually recover without residual deficits. Symptoms may last for several days and recovery takes days to occur.
* **EFFECTS OF SHORT-TERM (LESS THAN 8-HOURS) EXPOSURE**: Tetrodotoxin interferes with the transmission of signals from nerves to muscles by blocking sodium channels. This results in rapid weakening and paralysis of muscles, including those of the respiratory tract, which can lead to respiratory arrest and death.
* **INGESTION EXPOSURE**:
* First stage: Numbness and sensation of prickling and tingling (paresthesia) of the lips and tongue, followed by facial and extremity paresthesias and numbness, headache, sensations of lightness or floating, profuse sweating (diaphoresis), dizziness, salivation (ptyalism), nausea, vomiting (emesis), diarrhea, abdominal (epigastric) pain, difficulty moving (motor dysfunction), weakness (malaise), and speech difficulties.
* Second stage: Increasing paralysis, first in the extremities, then in the rest of the body, and finally in the respiratory muscles; difficulty breathing or shortness of breath (dyspnea); abnormal heart rhythms (cardiac dysrhythmias or arrhythmia); abnormally low blood pressure (hypotension); fixed and dilated pupils (mydriasis); coma; seizures; respiratory arrest; and death.
* **FIRST AID:** Initial treatment is primarily supportive as there is no antidote for TTX toxicity at this time. Remove the individual from the exposure source. Prevent others from eating until the source of TTX exposure can be ascertained. If ingested, DO NOT induce vomiting - seek medical attention immediately. For contact exposure to the eyes/face, use the eyewash to flush eyes/ with water for at least 15 minutes. For dermal exposure, rinse area with copious amounts of water for at least 15 minutes, remove any contaminated clothing.
* **REPORTING:** Submit an incident/injury report on the EH&S website <https://www.ehs.uci.edu/> or call 949-824-6200 within 8 hours. The report can be submitted on your behalf by the PI. In addition, notify the IBC (ibc@uci.edu).

*Reference: CDC/NIOSH:* <https://www.cdc.gov/niosh/ershdb/emergencyresponsecard_29750019.html>

**TECHNICAL PROCEDURES**

**Reconstitution, dilution, and administration of the toxin will ONLY be performed in a certified BSC while wearing PPE.**

[insert technical procedures associated with TTX. Procedures may include reconstitution, experiment set up for TTX treatment, decontamination of specialized equipment)

**Acknowledgement**

**As the Principal Investigator, it is your responsibility to ensure that all individuals in your laboratory are taught correct procedures for the safe handling of hazardous materials involved in this study. It is also your responsibility to assure that your personnel have completed all the required training. The PI and other individuals associated with the protocol must sign the following acknowledgement: *I have read, asked questions, and understand the hazards of and safe working procedures for the activity/materials described herein.***

PI Signature DATE

Other Personnel (add more rows as needed):

Name/ Signature DATE

Name/Signature DATE

Name/Signature DATE

Name/Signature DATE