Appendix E: Template for high hazard chemical work

HIGH HAZARD LAB OPERATIONS

WORK REQUIRING PRIOR APPROVAL AS DEFINED IN THE OSHA LAB STANDARD AND THE CHEMICAL HYGIENE PLAN

Principle Investigator: _____ Department: _____

Project Title:_____

Work Location(s) Building and room #: _____

Name and Titles of Personnel Approved/Trained for Procedure:

Describe the proposed work, be sure to include the following:

Use:
TTX is used to characterize sodium channels in excitable membranes and to study the role of sodium channels in normal physiology and disease. To inhibit voltage-gated sodium channels action potentials generated in mouse brain slices or cultured primary cell lines.

Hazard:
It is a neurotoxin, a selective sodium(Na) channel blocker that blocks propagation of impulses in excitable membranes. Reversible, selective blocker of Na^+channels; blocks propagation of impulses in excitable membranes.

Synonyms	Fugu poison
	TTX
	Maculotoxin
	Tarichatoxin
Molecular Formula	$C_{11}H_{17}N_3O_8$
Molecular Weight	319.27
CAS Number	4368-28-9
Beilstein Registry Number	49176
EG/EC Number	2244588
MDL number	MFCD00213719

Routes of Entry:

Very toxic by inhalation, in contact with skin and if swallowed.
Target organ(s): Nerves. Skeletal muscle.
HMIS RATING
HEALTH: 4* Ingestion: May be fatal if swallowed.
TARGET ORGAN(S) OR SYSTEM(S)
Nerves. Skeletal muscle. Tetrodotoxin is among the most toxic substances known to man.
Death can occur within 30 minutes. It is extremely dangerous by ingestion, inhalation and skin absorption, or if it enters the bloodstream in any manner. Symptoms of poisoning which include numbness, tingling of the lips and inner mouth surfaces, weakness, paralysis of the limbs and chest muscles, and a drop in blood pressure have been reported within as little as 10 minutes after exposure. To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.
Color: White
Form: Powder
Hazardous Decomposition Products: Carbon monoxide, Carbon/Nitrogen oxides.

Label Precautionary Statements:
Highly Toxic (USA) Very Toxic (EU).

Quantity and Concentration Purchased:
Manufacturer/Vendor or Source:
Ordering Information and Chain of Custody, and Storage
Vendor Information:
SigmaAldrich, P.O Box 14508, St. Louis, MO 63178 USA, 800-325-5832,

An order can be placed online from Sigma/Aldrich Chemicals. T5651-1mg Tetrodotoxin with Citrate Buffer is usually ordered at 5 of 1mg size per shipment in this laboratory. A Declaration of Aggregate Quantities Form is required to be signed by the principle investigator before an order is processed. A maximum of 100mg aggregated quantities is allowed per order. Inventory control is paramount, all orders must be approved by the PI so that at no time the lab exceeds 10mg of tetrodotoxin aggregate. The item is shipped via fedex overnight. The tetrodotoxin package is opened in a chemical hood with the personnel wearing safety goggle, laboratory coat & latex gloves. Then, the person who receives the package records the material onto the inventory sheet & store unopened packages in a locked refrigerator located inside the laboratory(Borwell/750E).

Typical Amount Used per Experiment:
A stock vial is prepared and shared with all users. 1mM concentration stock is prepared for use in electrophysiology patch clamping recording. One to one hundred microliters volume range of TTX is used per experiment, dependent upon total volume of the perfusion solution required for an acute slice preparation. A final concentration of 1uM is dissolved in saline or ACSF. A dedicated container is used to prepare TTX in perfusion

solution(ACSF). The waste perfusion solution is then collected into a large flask, which contains 10% Clorox solution. All the washes from the reservoir and slice chamber are collected into the labeled waste container. A log sheet is kept to track usage by approved and trained users.

Dose that causes adverse effects/symptoms:
0.32 µg/kg if administered subcutaneously (which is 24 µg in a 75 kg human)

Lethal dose
0.334 mg/kg in mouse via oral administration
8.0 µg/kg in mouse via subcutaneous administration
14.0 µg/kg in frogs via intraperitoneal administration

Symptoms of Exposure –
It is extremely dangerous by ingestion, inhalation and skin absorption, or if it enters the bloodstream in any manner. Symptoms of poisoning which include numbness, tingling of the lips and inner mouth surfaces, weakness, paralysis of the limbs and chest muscles, and a drop in blood pressure have been reported within as little as 10 minutes after exposure.

First Aid – What to do if exposed – Provide the MSDS/SDS or other first aid info to the EMT's/Medical care staff.
ORAL EXPOSURE
If swallowed, wash out mouth with water provided person is conscious. Call a physician immediately. Call 911
INHALATION EXPOSURE. Call 911.
If inhaled, remove to fresh air. If not breathing give artificial respiration. If breathing is difficult, give oxygen. Call 911.
DERMAL EXPOSURE
In case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. Call a physician. 911
EYE EXPOSURE
In case of contact with eyes, flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. Call a physician. Call 911.

Inspect the room immediately by a supervisor following spill & assess the degree of emergency.

Procedures for Use:

Safety precautions required when diluting TTX in the laboratory chemical fume hood, PPE - chemical resistant nitrile rubber gloves , goggles & lab coat.
-Researcher wears chemical resistant nitrile rubber gloves and chemical safety goggles and laboratory coats.

-Dissolve powder with saline in chemical fume hood, to avoid aerosolization of powder saline will be introduced to sealed vial, extract with a blunt cannula or hypodermic needle.
-Make small aliquots.
- Aliquots stored in container labeled highly toxic and stored in a locked refrigerator.
- Remove contaminated gloves or debris and place in hazardous waste or decontaminate by soaking in 10% bleach.
-Wash hands

Storage
We plan to store the TTX stock at 1mM in a dedicated container and in a designated area in the locked refrigerator in Borwell, 750E. Aliquots will be taken from the stock and diluted to the desired concentration immediately before each experiment.

Disposal
All excess ttx or stock solutions must be disposed via EHS. All waste materials will be properly labeled and safely stored inside a chemical hood until pickup.
After use, untensils(pipette tips, glass recording pipettes) should be soaked overnight or boiled for 20 minutes in an excess amount of 2% sodium hydroxide solution or sodium hypochlorite(1% available chlorine) solution in a hood. Also, observe all federal, state, and local environmental regulations.

Security and Restricted Access
The toxin is stored in a locked refrigerator in 750E laboratory. The key will be locked in a locked drawer within the laboratory. Only the trained users know where the key to the locked drawer is stored. There is an emergency contacts list posted outside of the laboratory. NO TRANSFER OR SHIPMENT OF TOXIN ALLOWED

Spill Clean-up – Contact EHS to report or for assistance 646-1762
Evacuate area. Only personnel on the emergency contact list can remain to assist in clean up. Spilled material should be carefully wiped or moistened with water and removed. Decontaminate any spill with 10% caustic solution Ventilate area and wash spill site after material pickup is complete. Remove contaminated clothing to be decontaminated by rinsing & washing thoroughly. The date & amount of spill & who was exposed should be documented on Toxin Inventory sheet. Also, report incident to supervisor & complete Incident Report form.

Emergency Contact Personnel and Telephone Numbers:

Review MSDS/SDS prior to handling of TTX. The MSDS/SDS is available from the supplier and/or on the EHS web site.

PI:
EHS:606-646-1762
DHMC Occupational Medicine: 603-653-3850
Safety and Security:603-646-2234

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Reccomended Special Practices

1) Identify the hazards that will be encountered under normal work conditions, and those that could be encountered in case of a spill, loss of containment or other accident.
2) Specify the policies and practices to be used to minimize risks (e.g., containment and personal protective equipment, management of spills, management of accidental exposures, medical surveillance).
3) Training specific to the hazards/chemicals used should be required and documented for all laboratory personnel working with these hazards, before starting work and at intervals thereafter.
4) An inventory control system for highly hazardous chemicals/substances should be in place and they should stored in locked storage rooms, cabinets, or freezers when not in use.
5) Access to areas containing high hazard materials should be restricted to those whose work assignments require access.
6) Preparation of primary containers of stock solutions and manipulations of primary containers of pure chemicals/toxins should be conducted in a chemical fume hood, a glove box, or a biological safety cabinet or equivalent containment system. HEPA and/or charcoal filtration of the exhaust air may be required, depending on the material.
7) The user should verify inward airflow of the hood or biological safety cabinet before initiating work.
8) All work should be done within the operationally effective zone (6-8" inside the hood face) of the hood or biological safety cabinet.
9) When high risk operations are underway, the room should be posted to indicate this "_____ in Use Authorized Personnel Only." Any special entry requirements should be posted on the entrance(s) to the room. Only personnel whose presence is required should be permitted in the room while high risk operations are ongoing.
10) All high risk operations should be conducted with two knowledgeable individuals present. Each must be familiar with the applicable procedures, maintain visual contact with the other, and be ready to assist in the event of an accident.
11) Before containers are removed from the hood, cabinet, or glove box, the exterior of the closed primary container should be decontaminated and placed in a clean secondary container. Hazardous materials should be transported only in leak/spill-proof secondary containers.
12) Contaminated and potentially contaminated protective clothing and equipment should be decontaminated using methods known to be effective against the material before removal from the laboratory for disposal, cleaning or repair. If decontamination is not possible/practical, materials (e.g. used gloves) should be disposed of as hazardous waste.
13. The interior of the hood, glove box, or cabinet should be decontaminated periodically, for example, at the end of a series of related experiments. Until decontaminated, the hood, box, or cabinet should be posted to indicate that hazards are in use, and access to the equipment and apparatus restricted to necessary, authorized personnel.

Safety Equipment

1. When using an open-fronted fume hood or biological safety cabinet, impervious protective clothing, including gloves and a disposable long-sleeved body covering (gown, laboratory coat, smock, coverall, or similar garment) should be worn so that hands and arms are completely covered.
2. Eye protection should be worn if an open-fronted containment system is used.
3. Other protective equipment may be required, depending on the hazards posed and and the containment system used.
4. When handling dry forms of hazardous materials that are electrostatic:

a. Do not wear gloves (such as latex) that help to generate static electricity

b. Use glove bag within a hood or biological safety cabinet, a glove box, or a class III biological safety cabinet.

5. When handling materials that are percutaneous hazards (irritants, necrotic to tissue, or extremely toxic from dermal exposure), select gloves that are known to be impervious to the chemical and any solvent carrier(s). This will often mean double gloving with silver shield laminate glove liners and heavy duty outer gloves.

Additional Information/Definitions:

Hazards: Describe the primary hazards posed by this work including chemical hazards (fire, explosion, water/air reactivity, high toxicity, corrosiveness or ability to generate toxic gasses) and physical hazards (high/low pressure or temperature, compressed gasses, UV or other harmful radiation).

Description of Safety Precautions and Equipment: Discuss location of work with attention to exposure control equipment as the primary method of protection. Note if you will be working iin a certified chemical fume hood or biological safety cabinet Class II type B2 or B3. Hood performance must be assessed before each use via a hood monitor or use of smoke/telltale to verify flow. Glove boxes or other advanced engineering controls may be required for significant hazards. Personal protective equipment (PPE) includes gloves, eye/face protection and other items including hearing protection. Engineering controls must be the primary method of protection but PPE also needs to be evaluated.

Security: Describe the inventory control, labeling and tracking methods for high hazard chemicals. Include provisions for securing storage locations and controlling access during work periods and off-hours.

Description of decontamination and waste disposal procedures: Provide specific information on types of waste generated and collection and disposal methods. Include provisions for contaminated debris, disposal of pure materials and stock solutions. If materials or spills are to be decontaminated for disposal in the trash describe the decontamination method to be used and provide references.

Description of emergency response and first aid requirements: Include information on any unique first aid treatments or provisions and emergency procedures for isolation/evacuation of the area.