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| PI: |  |  |
| Agent(s): | Tetrodotoxin - TTX |
| Date SOP Created: |  |

*Instructions: Insert specific details pertaining to your research and delete irrelevant procedures; contact EH&S at 642-3073 or OLAC at 642-9232 as needed for assistance.*

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| Hazard Information | *Work with tetrodotoxin requires a BUA and may only be performed in reserved, pre-approved locations on campus. Tetrodotoxin (TTX) is a Select Agent. Prior to use, verify that the quantity you require for research is below the restricted threshold by contacting the Campus Biosafety Officer at* [*bso@berkeley.edu*](mailto:bso@berkeley.edu)*. See* [*http://ehs.berkeley.edu/biosafety*](http://ehs.berkeley.edu/biosafety) *or contact EH&S at 642-3073 for authorization and biosafety training prior to beginning work.*  Tetrodotoxin (TTX) exposure by inhalation, swallowing, or by subcutaneous exposure is rapidly acting and extremely toxic. TTX blocks the voltage-gated sodium channel on the surfaces of nerve membranes, cardiac tissue and muscle tissue.  Avoid any exposure and wash hands thoroughly after handling. BSL-2  practices should be followed. Engineering controls such as fume hoods and biological safety cabinets must be used as primary containment in order to limit personnel exposure to TTX. Engineering controls must be inspected in order to ensure efficient removal of hazard, and must have a visual indication of airflow, and alarms to indicate that airflow has fallen below acceptable standards. It may be appropriate to wear N95 particle respirators when handling stock powder –  contact EH&S for advice.  The LD50 for humans, i.v., is 2-10 μg/kg. Oral ingestion of 1-2 mg has caused death in humans. LD50 for inhaled TTX in humans is 2μg/kg. The main lab risks for this toxin are exposure by direct contamination of mouth, eyes, or other  mucous membranes and by needle-sticks or other accident that may compromise the normal barrier of the skin. Signs and symptoms of TTX exposure include: burning/tingling of lips, face, extremities; vertigo or dizziness, weakness, nausea or vomiting, rapid onset of muscle spasm, and respiratory distress.  Hazards specific to your agent and route of administration: |
| Personal Protective Equipment (PPE) | when working with powder form  Non-permeable static free gloves  Long-sleeved lab coat  Safety glasses or goggles  Respiratory protection may be required if aerosols may be generated and it is not possible to use containment equipment or other engineering controls.  when working with TTX in solution  Non-permeable gloves  Long-sleeved lab coat  Safety glasses or goggles  when working with animals:  Disposable gown  Gloves  Safety glasses or goggles or face shield  Respiratory protection may be required if aerosols may be generated and it is not possible to use containment equipment or other engineering controls.  *Additional PPE specific to your research:* |
| Preparation | *List procedures used. Be specific about the physical form (solid, liquid, etc.) and locations for work (bench top, fume hood, biosafety cabinet), and personal protective equipment (PPE) to be worn when handling the material.*  TTX will be purchased/obtained from *(List provider)*. Package will be kept intact with shipping documentation and/or maintained in double containment with proper labeling, including PI name and contact information. Tetrodotoxin (TTX) will be purchased from suppliers in smallest amounts available (est 1mg). Inspect the containers for damage during shipment. If damaged, contact the vendor immediately, and decontaminate and dispose of the shipment immediately.  All handling of powder must be performed within a fume hood in *(List location)*. The fume hood will be posted as “Restricted Area -Toxin in Use”. Place an absorbent pad inside of the fume hood during handling. Powder will be resuspended by pipetting, using a filter tip. All dilutions will be made by individuals wearing required PPE listed above, in the presence of another trained individual. If aliquoting samples, resuspension of the toxin will be performed with extremely careful and slow titration, rinsing down the walls of the tube in the process avoiding foaming and aerosolization. Aliquots of 5 mLs of 10% bleach  solution into a 50 ml conical tube will be made and open tubes will be placed in a tube rack. This tube will serve as a waste receptacle for contaminated pipet tips. Toxin vials will be placed in an unbreakable, easily decontaminated, clean secondary storage container and transferred to storage at the appropriate temperature. TTX stocks will be stored in a clearly marked secondary container  labeled “Toxin. Do Not Handle”. All TTX inventory will be secured (locked up) and recorded in a bound notebook within the lab. Decontaminate all work surfaces and the exterior of all materials leaving the fume hood with 10% bleach before removing “Restricted Area” posting.  *Other specific preparation steps (with location):* |
| Transportation | TTX will be carried in an easily decontaminated, leak-proof, secondary container labeled with a biohazard symbol and PI name and contact information to *(list approved location)*. Avoid the use of glass containers. Handling will only be done by  trained personnel. |
| Use | Electrophysiology: Electrophysiology rigs *(list approved location)* will be posted with “Toxin in Use” sign during all work. Workers will wear Non-permeable gloves and arm protection (e.g., lab coat) at all times. Liquid wastes will be treated with bleach before disposal as chemical waste. Immediate work area will be cleaned with 10% bleach by user wearing nitrile gloves.  While working in *(list approved animal location)* with TTX and if handling animals within 48 hours post-infection, the following PPE must be worn: **Disposable Gown, Gloves, Face Shield (Disposable).**  *Description of infection procedures:*  *Example: “On the day of experiments, TTX will be diluted to a working concentration of 1 microM in a chemical fume hood. Mouse retinas will be incubated in artificial cerebral spinal fluid (ACSF) and then transferred to a recording bath where the slices will be perfused with TTX (1uM).”* |
| Disposal and Disinfection If unsure, contact EH&S at 642-3073 to determine disposal procedures. | * Avoid use of glass, if at all possible. Sharps with engineering controls should be used for injection. Glass and other sharps must be disposed of in a dedicated sharps container. * Liquid wastes are treated with bleach before disposal as chemical waste. * Gloves and other contaminated solids disposed of as chemical waste. * Animal carcasses will be disposed in red barrels as biohazardous/pathology waste. * Decontaminate all work surfaces and equipment with 10% bleach prior to removal of postings. * After infection, disposable cages may be returned directly to standard ABSL1 housing but the cage card (obtain from OLAC) must be labeled to indicate the hazard type, agent, date of administration, and that OLAC should not change the cage for 48 hours. After 48 hours, animals should be transferred to clean cages by the researcher within a functioning biosafety cabinet. Within the biosafety cabinet, used cages and bedding should be bagged within biohazard bags and disposed of as trace chemotherapy waste. |
| Spill Response and Emergency Procedures | Recommended disinfectants: 10% household bleach (>0.5% NaOCl for >30 min contact time)  *Disinfectant to be used:*  Injury: If eye or skin contact occurs, wash affected areas with copious amounts of water for 15 minutes and IMMEDIATELY seek medical advice. If inhaled, move individual to fresh air and IMMEDIATELY seek medical advice, call 911. [Rescue breathing, CPR may be needed.] If swallowed, seek IMMEDIATE medical advice.  Report the incident to your supervisor and the Occupational Health Clinic at 2-6891 for follow up.  Medical attention during normal business hours: Tang Center Urgent Care (2-3188 or 3-7197); after hours go to urgent or emergency care: Alta Bates Hospital at 2450 Ashby (204-4444).  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 should be:  • No specific antitoxin is available  • Supportive care  • Anticipation of progressive paralysis, particularly of the respiratory system  • Stomach evacuation by the emergency department if ingested  If a spill should occur: Avoid generating dust. With gloved hands, remove any contaminated clothing and put in red biohazard bag. Notify other workers in the area of the spill and control traffic through area. Wear shoe covers if the spill is on the floor. Put on fresh gloves and cover spill area with paper towels. Pour  10% bleach over towels from edges of spill to center; be careful not to splatter. Decontaminate all objects in spill area. Allow 30 minutes of contact time. Pick up any sharps, including broken glass, with forceps and place in sharps container. Wipe area with bleach and clean towels again, mop if the spill is on the floor. Remove gloves and foot covers before leaving area of the spill, put in biohazard bag, and wash hands. Monitor for signs of exposure.  For exposures or emergency clean up response, notify EH&S at 642-3073 immediately. All spills must be notified to EH&S within 8 working hours. |
| Hazard Communication (signs, cage cards, etc.) | All researchers handling this material must read and sign this document.  When toxins are in use, the doors will remain closed and the room should be posted to indicate “Toxins 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 toxins are in use. Postings will not be removed until all surfaces have been decontaminated with 10% bleach for 30 minutes.  After 48 hours of final treatment, animals should be transferred to clean cages by the researcher within a functioning biosafety cabinet and the “OLAC Do Not Change” card should be removed to indicate OLAC may resume care. Within the biosafety cabinet, used cages should be bagged within biohazard bags and disposed as trace chemotherapy waste.  EH&S and an OLAC veterinarian must review and date this SOP prior to starting this work within an animal facility. Obtain cage cards and hazard labels from OLAC. During injections this SOP must be posted in a plastic sleeve on the door of NAF 120F, LSA 640, Minor 599E *(choose location or list approved location)* to notify OLAC staff and other personnel. |
| Unique Instructions | Before you start work: Review hazards of toxin you are handling, complete required reading indicated below, sign and date below, rehearse all handling tasks with a placebo for practice, demonstrate worker proficiency to a supervisor and post signage. All high-risk tasks, such as work with stock material (i.e. making dilutions) must be conducted with two trained individuals present.  Coordinate use of NAF 120F, LSA 640, Minor 599E *(choose location or list approved location)* with the OLAC Facility Manager.  *Other unique procedures:* |
| Additional Information or References | Refer to applicable protocols and authorizations, e.g. the lab’s Biohazard Use Authorization, MAUP/eProtocol, SDS available at <http://ehs.berkeley.edu/hazardous-materials/safety-data-sheets-formerly-msds>, your lab’s chemical hygiene plan, or contact your supervisor or EH&S at 642-3073 for further guidance.  NIH/CDC BMBL Appendix I: Guidelines for Work with Toxins of Biological Origin:  <http://www.cdc.gov/OD/ohs/biosfty/bmbl5/BMBL_5th_Edition.pdf>  Good Work Practices with Toxins: <http://www.ehs.ufl.edu/bio/ToxinArt.pdf>  *Other required protocols or references:* |

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| **Print Name (last, first)** | **Signature** | **Date Plan Reviewed** |
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PERSONNEL SIGNATURES

EH&S Review (Name/Date):­ 642-3073

OLAC Representative Review (Name/Date): 642-9232