

Tetrodotoxin (TTX)

Tetrodotoxin is a neurotoxin from marine sources. It is a small molecular weight toxin (MW = 319). Tetrodotoxin is extremely potent, with a very rapid onset of symptoms.

General Safety considerations:

- Exposure to TTX can be rapidly fatal! Upon any exposure to any amount of TTX that could cause symptoms, call 911 immediately! If UW-Madison Office of Biological Safety (OBS) requires that you handle the TTX in containment, then it is a large enough amount to require calling 911 upon exposure.
- After exposure to TTX, the PI and supervisor MUST notify the UW-Madison Office of Biological Safety (OBS), UW-Madison Select Agent staff, and UW-Madison Occupational Medicine staff. A First Report of Exposure/Release MUST be submitted within 24 hours of an exposure to TTX.
- Exposure routes: Inhalation, oral, and parenteral (through the skin).
- Tetrodotoxin is a sodium channel blocker in nerve and muscle tissues. This leads to paralysis of nerve and muscle function, including muscles involved in breathing.
- Tetrodotoxin is generally acquired in powdered form. Dry forms of tetrodotoxin are **always** handled in a chemical fume hood or ducted certified biological safety cabinet **Be extremely careful** when handling any amount of tetrodotoxin in dry form. Only order as much as you need to use in the immediate future and resuspend the entire vial at once rather than weighing out aliquots.
- TTX is unusually resistant to steam autoclaving and inactivation by 10% aqueous bleach solution.

Biosafety Protocol

At UW-Madison, research laboratories that utilize purified Tetrodotoxin in their research programs must list information about use of the toxin in their Bio-ARROW protocol. The UW-Madison Institutional Biosafety Committee will review all protocols that include use of Tetrodotoxin, and review will be in the closed session portion of the meeting because of its status as a Select Agent Toxin. Following is information about adding Tetrodotoxin to specific sections of the Bio-ARROW protocol:

Select Agents

- The Federal Select Agent Program does not regulate certain Select Agent (SA) toxins if the amount under the control of a PI does not exceed, at any time, the amounts indicated on their website (<https://www.selectagents.gov/permisibletoxinamounts.html>). UW-Madison requires that PIs maintain an accurate inventory of SA toxins and secure the toxins in their laboratory. Inventory reports are submitted semi-annually (every 6 months) to the Office of Biological Safety (biosafety@fpm.wisc.edu).

- The permissible amount of TTX that an investigator can possess in their laboratories without registering as a Select Agent laboratory is 500 mg.
- Select Agent Principal Investigator (PI):
 - If the PI of the protocol is a Select Agent registered person, please contact the UW-Madison Select Agent Program for guidance about listing TTX on the protocol.
 - If the PI of the protocol is not a Select Agent registered person, select “No”.

Microbes

- Microbes treated with tetrodotoxin are handled at BSL2 or (if applicable) BSL3.

Biological Toxins: Biological Toxins Details

- Select Agent: Yes
- Botulinum Toxin: No
- Biological Toxin Select Agent Inventory: Please indicate that toxin is kept in a secured fridge or freezer, a current inventory is maintained, and semi-annual (every 6 months) inventory reports are submitted to the Office of Biological Safety at biosafety@fpm.wisc.edu.
- Amount: Estimated maximum total amount of tetrodotoxin you will have in your laboratory. Note that the total amount of tetrodotoxin under the control of the PI at any time must not exceed 500 mg.
- Form: Enter all forms handled (dry/lyophilized, aqueous, etc.) and note if the dry/lyophilized form is only handled for reconstituting.
- LD₅₀: 8 ug/kg (IV, mouse); 334 ug/kg (oral, mouse).
- Biosafety Level: BSL2
- Storage only: Select “yes” only if all of the TTX in the laboratory is now remaining in storage and is not in active use in your research program.

Cells, Organs, Tissues, or Biological Specimens

- Cells and tissues are administered tetrodotoxin in a biological safety cabinet at BSL2. Requests to handle TTX outside of containment must be reviewed by the Office of Biological Safety and approved by the IBC.
- Treated cells or tissues are subsequently handled at BSL2.

Vertebrate Animals

- ABSL2 housing is adequate for most animal studies involving TTX. OBS staff will work with labs to determine the required biosafety level for these animals, and whether administration of TTX to animals must occur in a BSC.

Containment

- Aerosol Generating Activities: Cage changes of rodents treated with TTX must be performed in a biological safety cabinet.

PPE

- Lab coat, eye protection, and disposable gloves must be worn when handling TTX.
- Depending on the research activities being performed with TTX, a fit-tested respirator (N95, etc.) may also be required.

Disinfection and Inactivation - Animal

- Because tetrodotoxin could potentially be present in bedding of tetrodotoxin-treated animals bedding must be treated to inactivate tetrodotoxin prior to disposal. A minimum of 20% bleach for at least 30 minutes is needed to inactivate tetrodotoxin.
- Carcasses of animals injected with tetrodotoxin should not need to be autoclaved prior to pick-up for incineration unless they need to be autoclaved for a different reason (also infected with a Risk Group 3 pathogen, for instance).

Disinfection and Inactivation - General: Biotoxins

- Tetrodotoxin is resistant to many standard methods of inactivation used for other biological toxins, such as autoclaving or treatment with 10% aqueous bleach. However, use of 20% aqueous bleach for at least 30 minutes should be sufficient to inactivate the toxin.
- Because of the potential aerosol risk, spills of tetrodotoxin outside of containment must include evacuation of the lab for at least 30 minutes to allow dissipation of aerosols.
- Because the microwave inactivation process utilized at MERI may be insufficient to inactivate tetrodotoxin, materials being sent to MERI that may contain tetrodotoxin must first be treated to inactivate any tetrodotoxin present.

Spill and Release Procedures:

- Please note that the Biosafety in Microbiological and Biomedical Laboratories (BMBL), current edition, recommends the following PPE are worn during a cleanup for a liquid toxin spill: mask, gloves, safety glasses or goggles and laboratory coat.

Signage

- A “Toxins in Use” sign is posted on the laboratory door when tetrodotoxin is being handled. The sign can be removed when no toxin is in use.
- Cage cards must specify that animals have been treated with tetrodotoxin.

Emergency Response

- UW-Medicine Occupational Medicine provides Medical Response plans for biological toxins and infectious agents through a link at <https://ehs.wisc.edu/workplace-safety/occupational-medicine-2/>.
- Emergency Response - General: Depending on the amount involved, exposures to tetrodotoxin could potentially lead to development of symptoms within minutes. Symptoms after exposure could include respiratory arrest or death. Thus, the first response to a TTX exposure is typically to call 911. Also, immediately wash or flush the affected area with soap and water for 15 minutes. Use an eyewash for 15 minutes after a splash to the eye. After a needlestick, immediately remove gloves and “bleed out” the

wound under running water for 15 minutes. PI or supervisor must submit a First Report of Exposure/Release form as soon as possible, and within 24 hours.

- Upon adding tetrodotoxin to your biosafety protocol, OBS personnel will conduct a risk assessment of the details of the proposed TTX usage (amount utilized, etc.) and will provide specific handling recommendations, including emergency response.

Laboratory Training

- Individuals handling tetrodotoxin or working in a laboratory where TTX is being handled must receive training about potential risks from exposure to the toxin as well as safe handling of the toxin as outlined in the biosafety protocol.
- Individuals handling TTX must receive training about security and inventory requirements for TTX.
- A separate spill protocol specifically for TTX may be required

Research Description

- Briefly describe what you will be doing with tetrodotoxin, including the amount that may be utilized at any one time. Specify the form of tetrodotoxin handled (typically aqueous).
- Specify the location and biosafety level for research involving TTX.

Contacts and Additional Information

The sources listed may provide additional information about safe use of tetrodotoxin in research laboratories at UW-Madison:

- Office of Biological Safety (OBS); biosafety@fpm.wisc.edu, 608-263-2037
- Chemical Safety Department; chemsafety@fpm.wisc.edu, 608-265-5700
- Occupational Medicine; occmmed@uhs.wisc.edu, 608-265-5610
- First Report of Exposure or Release Form; <https://ehs.wisc.edu/first-report-of-biological-exposure-or-release-event/>
- Bio-ARROW KnowledgeBase; <https://kb.wisc.edu/arrow/ibc/page.php?id=43188>
- UW-Madison Select Agent Program; <https://www.selectagents.gov/sat/permissible.htm>; 608-890-4951
- Wannamaker, RW. Procedures for the Inactivation and Safe Containment of Toxins.