

## Tetanus neurotoxin (TeNT)

*Tetanus toxin (TeNT) is an extremely potent neurotoxin produced by Clostridium tetani. It is a large molecular weight molecule (150,000 kDa) that is very similar to botulinum toxin. Depending on the type of exposure, development of life-threatening muscle contractures can develop within hours or days of exposure. However, symptom development in under an hour could be possible with parenteral exposure to concentrated toxin. Potential exposure routes in a laboratory environment include cuts or punctures through the skin but TeNT may also be toxic through other exposure routes.*

### General Safety considerations:

- After exposure to TeNT, the PI and supervisor MUST notify the UW-Madison Office of Biological Safety (OBS) and UW-Madison Occupational Medicine staff. A First Report of Exposure/Release MUST be submitted within 24 hours of an exposure to TeNT.
- There are no known fatal laboratory exposures to TeNT. Although extremely potent, the toxin can be safely handled with minimal risk if all of the handling procedures outlined in the laboratory's Biosafety Protocol are stringently followed.
- Exposure routes: Inhalation, oral, and parenteral (through the skin).
- Parenteral (through the skin) is the most dangerous type of laboratory exposure to TeNT.
- In addition to being a neurotoxin, TeNT is classified by OSHA as a highly toxic chemical.
- TeNT blocks the exocytosis of neurotransmitters from motor neurotoxins. This leaves to a spastic paralysis, essentially a continuous spasm of affected muscles. Note that this is different than botulinum toxin, which causes flaccid paralysis of affected muscles.
- TeNT is generally acquired by laboratories in powdered form. Dry forms of TeNT are **always** handled in a chemical fume hood or ducted certified biological safety cabinet. **Be extremely careful** when handling any amount of TeNT in dry form. Only order as much as you need to use in the immediate future. Resuspend the entire vial at once rather than weighing out aliquots of dry toxin.
- TeNT is sensitive to heat and many chemical methods of inactivation. As a large protein toxin, it is readily inactivated by autoclaving or treatment with 10% bleach solution (0.5% sodium hypochlorite) for at least 30 minutes.

### Special considerations

- TeNT is produced by the *Clostridium tetani*. Under specific conditions, exposure to spores of these *C. tetani* in a deep wound could lead to TeNT production and subsequent absorption of the toxin within the anaerobic wound. The resulting disease is referred to as tetanus and is a significant safety consideration for laboratories handling these strains of bacteria.
- It is highly recommended that all personnel handling TeNT have a current immunization for tetanus toxin (Tdap, DTap, etc.).

## **Biosafety Protocol**

*At UW-Madison, research laboratories that utilize purified TeNT in their research programs must list information about use of the toxin in their Bio-ARROW protocol. Following is information about adding TeNT to specific sections of the Bio-ARROW protocol:*

### Select Agents

- Select Agent Principal Investigator: Select “No”. Although extremely potent, tetanus toxin is not a Select Agent Toxin.

### Recombinant Materials

- Experiments involving genes coding for fully toxic TeNT are considered a Major Action under Section III-B of NIH Recombinant DNA Guidelines. These experiments require prior approval by NIH.
- Experiments involving genes coding for smaller portions of TeNT, such as heavy chain or light chain, aren’t considered a Major Action by NIH. However, these experiments must be reviewed and approved by the UW-Madison IBC.

### Microbes

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

### Biological Toxins: Biological Toxins Details

- Select Agent: No
- Botulinum Toxin: No
- Biological Toxin Select Agent Inventory: Not Applicable
- Amount: Estimated maximum total amount of TeNT you will have in your laboratory.
- Form: Enter all forms handled (dry/lyophilized, aqueous, etc.) and note if the dry/lyophilized form is only handled for reconstituting.
- LD<sub>50</sub>: human, 3 ng/kg parenteral.
- Biosafety Level: BSL2
- Storage only: Select “yes” only if all of the TeNT in the laboratory is currently in storage and is not in active use in your research program.

### Cells, Organs, Tissues, or Biological Specimens

- Cells and tissues are treated with TeNT in a biological safety cabinet at BSL2. Requests to handle any amount of TeNT 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 TeNT. OBS staff will work with labs to determine the required biosafety level for these animals, and whether administration of TeNT to animals must occur in a BSC.

- Note that because TeNT is a very large protein toxin, rodents treated parenterally (IV, IP, subcutaneous, IM) with TeNT will not excrete TeNT in their urine. Thus, bedding for injected rodents should not contain TeNT. However, animals treated orally with TeNT potentially may excrete the toxin in their feces, and thus it might be present in the bedding.

#### Containment

- Aerosol Generating Activities: Unless handling of very small amounts of TeNT are approved by the IBC, the toxin must be handled within a Biological Safety Cabinet (BSC).
- Aerosol Generating Activities: Cage changes for animals treated parenterally with tetanus toxin does not require a BSC unless needed for a separate reason (also infected with a pathogen, for instance).

#### PPE

- At minimum, lab coat, eye protection, and disposable gloves must be worn when handling TeNT or TeNT-treated materials.
- Depending on the research activities being performed with TeNT, a fit-tested respirator (N95, etc.) may also be required.

#### Disinfection and Inactivation - Animal

- Because tetanus toxin is a large protein toxin, rodents treated parenterally (IV, IP, subcutaneous, IM) with tetanus toxin should not excrete the toxin in their urine. Thus, bedding for injected rodents should not contain tetanus toxin. However, animals treated orally with tetanus toxin potentially may excrete the toxin in their feces, and thus it might be present in the bedding. If bedding may contain tetanus toxin, then bedding should be autoclaved prior to disposal.
- Carcasses of animals injected with tetanus toxin 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

- TeNT is inactivated by autoclaving or by treatment with 10% aqueous bleach solution for 30 minutes.
- Because of the potential aerosol risk, spills of TeNT must include evacuation of the lab for at least 30 minutes to allow dissipation of aerosols.

#### 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 must be posted on the laboratory door when TeNT is being handled. The sign can be removed when no toxin is in use.
- Cage cards must specify that animals have been treated with TeNT.

#### Occupational Health Considerations

- It is highly recommended that all personnel handling TeNT have a current immunization for tetanus toxin (Tdap, DTap, etc.).

#### 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: In the event of exposure to TeNT, 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. Consult UW-Madison Occupational Medicine or the UW Hospital Emergency Department after any exposure to any amount or type of TeNT. Report exposures as soon as possible to the PI and/or laboratory supervisor. PI or supervisor must submit a First Report of Exposure/Release form as soon as possible, and within 24 hours.
- If concerned about potential rapid effects from exposure to TeNT, call 911.
- Upon adding TeNT to your biosafety protocol, OBS personnel will conduct a risk assessment of the details of the proposed TeNT usage (amount utilized, etc.) and will provide specific handling recommendations, including emergency response.

#### Laboratory Training

- Individuals handling TeNT or working in a laboratory where TeNT is being handled must receive training about potential risks from exposure to the toxin as well as safe handling methods as outlined in the laboratory’s biosafety protocol.
- A separate spill protocol specifically for TeNT may be required.

#### Research Description

- Briefly describe what you will be doing with TeNT, including the amount that may be utilized at any one time. Specify the form of TeNT handled (typically aqueous).
- Specify the location(s) and biosafety level(s) for research involving TeNT.
- Staff from the Office of Biological Safety (OBS) will review the potential exposure risks for research activities proposed with TeNT and may require additional PPE or modified handling practices prior to the start of the research.

#### **Contacts and Additional Information**

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

- Office of Biological Safety (OBS); [biosafety@fpm.wisc.edu](mailto:biosafety@fpm.wisc.edu), 608-263-2037
- Chemical Safety Department; [chemsafety@fpm.wisc.edu](mailto:chemsafety@fpm.wisc.edu), 608-265-5700
- Occupational Medicine; [occmmed@uhs.wisc.edu](mailto: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=43082>