

## Diphtheria Toxin (DT)

*Diphtheria toxin is an extremely potent exotoxin produced by *Corynebacterium diphtheriae*. DT is a large molecular weight (70,000) protein consisting of two subunits, A and B.*

### General Safety considerations:

- There have not been any known fatal lab exposures to diphtheria toxin. 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, ingestion, and parenteral (through the skin).
- After exposure to DT, 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 any exposure to DT.
- Diphtheria toxin is classified by OSHA as a highly toxic chemical.
- DT enters the cytoplasm of cells and inhibits protein synthesis, leading to cell death. It can cause a variety of symptoms within hours, including myocarditis, kidney failure, and neuropathy. Death can occur from heart and liver necrosis.
- Diphtheria toxin is generally acquired in powdered form. Dry forms of diphtheria toxin are **always** handled in a chemical fume hood or ducted certified biological safety cabinet. **Be extremely careful** when handling any amount of diphtheria toxin 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.
- As a large protein, DT 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

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

### Biosafety Protocol

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

#### Select Agents

- Diphtheria toxin is not a Select Agent Toxin.

#### Recombinant Materials

- Experiments involving genes coding for fully toxic DT 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 DT such as the A-subunit (DT-A), 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 diphtheria toxin 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 diphtheria toxin 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.
- LD50: 0.1 ug/kg (IM, human)
- Biosafety Level: BSL2
- Storage only: Select "yes" only if all of the DT 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 DT in a biological safety cabinet at BSL2. Requests to handle any amount of DT 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 DT. OBS staff will work with labs to determine the required biosafety level for these animals, and whether administration of DT to animals must occur in a BSC.
- Note that because DT is a very large protein toxin, rodents treated parenterally (IV, IP, subcutaneous, IM) with DT should not excrete DT in their urine. Thus, bedding for injected rodents should not contain DT. However, animals treated orally with DT 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 DT 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 diphtheria toxin does not require a BSC unless needed for a separate reason (also infected with a pathogen, for instance).
- Aerosol Generating Activities: Cage changes for animals treated orally with DT must occur in a BSC.

### PPE

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

### Disinfection and Inactivation - Animal

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

- DT is inactivated by steam autoclaving or by treatment with 10% aqueous bleach solution for 30 minutes.
- Because of the potential aerosol risk, spills of DT 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 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 DT.

### Occupational Health Considerations

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

### Emergency Response

- UW-Madison 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 DT, 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 ASAP after any exposure to any amount or type of DT. 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.
- Note that during relevant emergency response for labs conducting research with the A subunit of DT (DT-A), it is important to specify to everyone involved (medical care providers, etc.) that the toxin involved is the less toxic A subunit and not highly toxic diphtheria toxin.

### Occupational Health Considerations

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

### Laboratory Training

- Individuals handling DT or working in a laboratory where DT 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.
- A separate spill protocol specifically for DT may be required.

### Research Description

- Briefly describe what you will be doing with DT, including the amount that may be utilized at any one time. Specify the form of DT handled (typically aqueous).
- Specify the location(s) and biosafety level(s) for research involving DT.
- Staff from the Office of Biological Safety (OBS) will review the potential exposure risks for research activities proposed with PTX 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 diphtheria toxin 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=43188>