

**Duke University / Duke University Health System Policy on working with:  
Diphtheria Toxin**

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This policy was first approved on December, 2018.

**Occupational and Environmental Safety Office (OESO)  
Employee Occupational Health and Wellness (EOHW)**

**I. Revision Page**

<b>Date</b>	<b>Revision(s) / Comment(s)</b>
12/06/2018	New policy document

## II. Background

Diphtheria toxin (DT) is a biological toxin and is secreted by the bacterium *Corynebacterium diphtheriae*. DT is useful in biomedical research using mice because it can be used to selectively target and kill cells or organs without requiring surgery. Wild type mice do not have DT receptors, so they are relatively resistant to DT. The median lethal dose (LD<sub>50</sub>) for mice has been estimated at 1.6 mg/kg by subcutaneous injection compared to an estimated human LD<sub>50</sub> of less than 100 ng/kg by IM injection. The gene for DT receptors can be inserted into a mouse genome so that the transgenic mice will express DT receptors only on specific cells. For example, a transgenic mouse engineered to express DT receptors only on hepatocytes can be injected with DT, which will only kill the hepatocytes, creating a nonsurgical mouse model without a functional liver.

**Diphtheria toxin doses used in transgenic mice range from 0.5 µg/kg to 50 µg/kg depending on the scientific goal (10 to 1000 ng for a 20-gram mouse).[Saito et al, 2001; Cha et al, 2003; Cerpa et al, 2014] A common dose is 100 ng per injection, sometimes administered in repeated doses to achieve a cumulative effect. [Buch et al, 2005; Mann et al, 2016] This dose corresponds to  $\geq 0.014$  human LD<sub>50</sub>. Diphtheria toxin is commonly administered to mice in volumes ranging from 0.1 to 0.3 mL by intravenous, intraperitoneal, and intratracheal routes. [Mann et al, 2016]**

Humans are very susceptible to DT. It causes damage to the body by destroying cells or disrupting normal cellular metabolism. DT inhibits protein synthesis by catalyzing ADP-ribosylation of eukaryotic aminoacyltransferase II. DT is expressed by strains of *Corynebacterium diphtheriae*, which, are themselves infected with a bacteriophage that inserts the gene for toxin production. Not all strains of *C. diphtheriae* elaborate toxin, and non-toxigenic strains cause milder forms of infection. When humans are infected with *C. diphtheriae* bacteria, the toxin elaboration in the throat causes tissue sloughing which is known as trench mouth. If the infection persists, toxin expressed distant from the original entry site (usually the mouth/throat) attacks cardiac, nerve and kidney cells among others. The toxin, whether it is from an infection with *C. diphtheriae* bacteria or from an accidental exposure to the toxin alone, can cause myositis, arrhythmias, neuropathy, paralysis, kidney failure and even death.

Toxins such as diphtheria toxin are not infectious, do not replicate, and are not transmitted person to person, except by direct contact with the agent.

Accidental inoculation with the toxin leads to invasion of cells over a course of hours. The inflammatory response in the body to remove these dead cells takes days to weeks, and is the cause of illness and potentially death as a result of DT inoculation. During this time, monitoring and supportive care is the main source of treatment. For diphtheria patients, the risk of complications increases with each day/hour as toxin is absorbed. After about 3 days the ability of antitoxin to prevent complications (myocardial or neurologic) is markedly reduced as the toxin would have combined irreversibly with the tissue. However, that does not mean that antitoxin (DAT) should not be administered to neutralize remaining uncombined toxin. The antitoxin should be administered as soon as it is made available.

Diphtheria toxoid (inactivated toxin) is used for vaccination. Diphtheria toxoid is included in the pediatric (DT and DTap) and adult (Td and Tdap) vaccinations. Unvaccinated adults can be protected by three vaccine doses using Td or Tdap. The recommended intervals for adult vaccination are 4 weeks between the first and second dose, and 6 to 12 months between the second and third dose. [CDC, 2015]

### III. Risk Assessment

The medial lethal dose (LD<sub>50</sub>) for diphtheria toxin in humans has been estimated at < 0.1 µg/kg by the intramuscular route. [Gill, 1982; Barksdale et al, 1960] This translates to < 7 µg for a 70kg person.

Control strategies have been developed for high-risk and standard-risk research activities.

High Risk Diphtheria Toxin work is defined as the following:

1. Work with powder; diluting and/or aliquoting concentrated stock solutions
2. Working with a needle/syringe or other sharps with  $\geq 2$  microgram of toxin per sharps device
3. Working without sharps with tubes containing  $\geq 20$  micrograms of toxin per tube
4. Other procedures determined to be high risk by OESO

Standard Risk Diphtheria Toxin work is defined as the following:

1. Working with a needle/syringe or other sharps with < 2 microgram of toxin per sharps device
2. Working without sharps with tubes containing < 20 micrograms of toxin per tube
3. Other procedures determined to be standard risk by OESO

As noted earlier, not all experiments with DT require injecting high doses of DT into an animal or require working with high concentrations of DT in cell culture. Therefore, the risk of working with DT must be separated into two stages to apply the appropriate risk mitigation controls.

Stage 1 involves the preparation of the DT stock solution. DT is usually available from vendors in vials containing 1 mg lyophilized DT ( $\geq 142$  human LD<sub>50</sub>). Each vial is typically diluted in 1mL of diluent such as PBS. This is a high-risk procedure that uses a needle to inject the diluent through the septum of the vial. The highly concentrated stock solution (1 mg/mL) may be further diluted to a working concentration by the laboratory. For purposes of this policy, high risk work and standard risk work with DT are defined as noted above.

Stage 2 involves injecting the DT into animals, working with the DT in cell culture, etc. Each of these procedures have varying levels of risk. This risk is increased further when a sharp such as needle is used for injecting DT into animals and the amount of DT exceeds 2 µg/ syringe.

Since the risk profile varies based on specific experimental conditions, control strategies using **2µg/device of DT as the threshold when working with sharps and 20µg/per container without sharps** have been implemented for high-risk and standard-risk procedures (Section IV. Policy Statement). For a detailed description of working with DT—from preparing the stock solution to working with it in animals—the user must refer to laboratory-specific Standard Operating Procedures (SOP) developed in consultation with and approved by the OESO Biological Safety Division and Laboratory Safety Division (Appendix II).

**IV. Policy Statement**

Employees working with ‘Standard Risk’ and ‘High Risk’ quantities of Diphtheria Toxin (DT), as discussed in the Risk Assessment section, are required to follow the control strategies listed below as applicable. In general, to avoid accidental autoinoculation, extreme care must be exercised when handling DT in conjunction with any injection device.

All work with DT requires EOHW clearance as described in the table below. Serologic antibody monitoring is required for High Risk work.

In order to limit the number of personnel that must be cleared by EOHW for High Risk work with DT, it is recommended that the Principal Investigator (PI) identify one individual as the primary individual responsible for completing the reconstitution and dilution into standard risk work quantities. For practical matters and to ensure availability of a laboratory member who is medically-cleared to perform this task, it is also recommended that another individual from the laboratory is identified as a back-up. After the designated individual(s) perform Stage 1 tasks, others in the laboratory can work with the standard risk quantities.

It is the responsibility of the PI to appropriately identify and maintain both the primary and back-up personnel for the laboratory. It is also the responsibility of the PI to train the primary, back-up and all other personnel working with high and standard risk quantities of DT according to the OESO-approved Standard Operating Procedure (SOP).

Note: Any variance from these requirements will require a review and approval by the Biosafety Officer (BSO). The BSO may consult experts within OESO, EOHW and outside entities, as needed, to perform a risk assessment for this review and approval.

The control strategies are as follows:

<i>Medical Clearance/Surveillance Controls</i>		
	<b>Standard-Risk Quantities</b>	<b>High- Risk Quantities</b>
Medical Clearance	Clearance required by EOHW. Contact EOHW at 919-684-3136, option 2 to schedule an appointment.	
Vaccination Requirements [CDC, 2015]	1. Documentation of the receipt of a diphtheria-containing vaccine in the past 10 years.* 2. Unvaccinated adults will be required to receive additional doses of Tdap or Td to complete the 3-dose series for adults. 3. Boosters with Td or Tdap every 10 years.  *When documentation of vaccination is not available, a booster will be administered. If employee is a high-risk worker, a baseline antibody titer will be measured. Work restrictions will be placed on high-risk work until diphtheria toxoid IgG titer is protective (> 0.1 IU/ml).	

Serologic Surveillance	None	<ol style="list-style-type: none"> <li>1. Diphtheria toxoid IgG antibody must be over 0.1 IU/ mL prior to beginning research. This is the recommended level for adequate protection.</li> <li>2. Annual Diphtheria toxoid IgG antibody testing required. <ol style="list-style-type: none"> <li>a. Td or Tdap booster required if diphtheria toxoid IgG antibody falls below 0.1 IU/ mL.</li> <li>b. Testing is repeated 4 weeks after each booster.</li> </ol> </li> </ol>
<b><i>Administrative/Facility Controls</i></b>		
	<b>Standard-Risk Quantities</b>	<b>High- Risk Quantities</b>
Working with the lyophilized powder form	DT in powder form must never be handled in an open container outside of a biosafety cabinet or chemical fume hood.	
Transporting the lyophilized powder (within Duke; <i>not</i> shipment outside of Duke)	Personnel must transport vials/tubes containing lyophilized powder of DT in a secondary container to avoid dispersion if the vial/tube breaks during transport and movement outside of a biosafety cabinet or chemical fume hood.	
Work hours	No restrictions.	All work with high-risk concentrations must happen during business hours on a weekday (non-holiday). This includes preparing the stock solution, working with animals, tissue culture, etc.
Security	DT in solution must be kept securely and access ensured only to authorized, trained individuals.	DT in powdered form and in solution <b>must be locked</b> in a secure location and access ensured only to authorized, trained individuals.
Hand-washing sink; Eyewash/drench hose station	<p>A hand-wash sink must be readily available at all locations where DT is used. Hands must be washed thoroughly after removing gloves.</p> <p>Eyewash/drench hose station must be available for any splash injuries.</p>	

<i>Animal Work Controls</i>		
	<b>Standard-Risk Quantities</b>	<b>High- Risk Quantities</b>
Personnel working with animals	All personnel working with animals must be appropriately trained and receive the necessary clearances before working with animals.	
Animal sedation	Animal sedation is not required, however, appropriate physical or chemical restraint is recommended, as applicable for animal species, site of injection and complexity of injection procedures.	Personnel must sedate and tape in place the animal being injected with DT. (It is the PI's responsibility to receive appropriate IACUC approvals). Personnel must <u>not</u> hold animal in their hands while injecting with DT.
Animal handling during injection	To avoid accidental autoinoculation, extreme care must be exercised when handling DT in conjunction with any injection device. Personnel must ensure that the hand used for holding the animal is placed carefully to avoid accidental injections. Where feasible, a mechanical device such as a rubber-tipped forceps should be utilized to keep hands away from the injection site.	Personnel must <u>not</u> hold animal in their hands while injecting with DT. If necessary, a mechanical device such as a rubber-tipped forceps must be utilized to keep hands away from the injection site while still affording some level of control over the injection site.
Dosage per syringe	Based on risk assessment, each syringe may contain more than one dose for administration into more than one animal. Duke Biological Safety Division must review and approve this. (It is the PI's responsibility to receive appropriate IACUC approvals, as applicable.)	Each syringe must contain <i>only one</i> dose for <i>one</i> animal.
Syringe re-use	Same as above, see "Dosage per syringe." Additionally, in between re-use, syringe must be kept in a safe location, away from the operator, with the needle end of the syringe covered from accidental exposure to personnel (for example, the needle/syringe is placed inside an immobilized 50ml polypropylene tube taped to the work surface).	Personnel must <u>not</u> reuse syringes/sharps. Sharp safety devices must be activated immediately and the sharps must be disposed of immediately in a sharps box kept at the procedure site.



Total volume per syringe	Same as above, see “Dosage per syringe.”	
Safety-engineered sharps	Safety-engineered sharps must be used for animal injection.	
<b><i>Work Practice Controls</i></b>		
	<b>Standard-Risk Quantities</b>	<b>High- Risk Quantities</b>
Sharps disposal	Safety device must be immediately activated* and sharps must be disposed of immediately in a sharps box kept at the procedure site.  *exception as noted above for standard-risk quantities in “Syringe re-use”.	
Waste Disposal	All DT solutions will be handled using disposable plastic ware, which will be treated as biohazard waste after use. Reusable items must be autoclaved to inactivate DT.	
<b><i>Engineering Controls</i></b>		
	<b>Standard-Risk Quantities</b>	<b>High- Risk Quantities</b>
Biosafety Cabinet / Chemical Fume Hood Use	All aerosol & splash producing procedures and dilutions must happen in a certified biosafety cabinet or chemical fume hood.	All <b>reconstitution</b> , aerosol & splash producing procedures and dilutions must happen in a certified biosafety cabinet or chemical fume hood.
Needle Use	Personnel must minimize the use of needles as much as possible and substitute with other devices, as applicable. Safety-engineered sharps must be used for animal injection.	Personnel must <b>eliminate</b> the use of needles where possible and substitute with other devices as applicable. Safety-engineered sharps must be used for animal injection. Additionally, refer to Appendix II for specific procedures regarding reconstituting DT from lyophilized powder.
<b><i>Personal Protective Equipment (PPE) Controls</i></b>		
	<b>Standard-Risk Quantities</b>	<b>High- Risk Quantities</b>
Minimum PPE requirements	Laboratory: Lab coat and gloves. Safety glasses and mask for aerosol or splash generating procedures outside of the biosafety cabinet or chemical fume hood. Animal facility: ABSL1/ABSL2 PPE as per DLAR requirements.	
<b>Double</b> gloves	Not required.	Recommended.

## **V. Emergency Information**

1. In the event of an acute injury or overt exposure, the injured employee/student must immediately inform their supervisor/PI and notify Duke Employee Occupational Health and Wellness (EOHW) via the BBF (Blood Body Fluid) Exposure Hotline at (919)-684-8115 or 115 from a Duke Phone. EOHW may instruct the employee/student to report to the Duke Emergency Department (ED) for medical assessment and to take a copy of their laboratory protocol document to the ED including information about the dose associated with exposure.
2. The ED will initiate the post-exposure protocol and consult Duke Employee Occupational Health and Wellness (EOHW).

## **VI. Appendix I: EOHW Exposure/Injury Response Protocol**

See attached Appendix I for EOHW Exposure/Injury Response Protocol.

Duke Employee Occupational Health and Wellness (EOHW) has developed an Exposure/Injury Response Protocol to serve as the medical guidance document to assist EOHW and Duke ED staff in minimizing employee risk in the event of an accidental exposure or injury with Diphtheria Toxin (DT). It contains first aid instructions for personnel working with DT as well as for medical personnel responding to an exposure/injury to DT. It is the responsibility of the Principal Investigator (PI) to ensure that all laboratory personnel working with DT or could potentially be exposed to/injured by DT are appropriately trained in these procedures. See Section IV. Policy Statement for medical surveillance and vaccination requirements.

## **VII. Appendix II: OESO Standard Operating Procedure (SOP) Template**

See attached Appendix II for OESO Standard Operating Procedure (SOP) template.

Duke Occupational and Environmental Safety Office (OESO) Division of Biological Safety and Division of Laboratory Safety has developed the template for use by laboratories that work with Diphtheria Toxin (DT). OESO will make the template available as a separate editable document on its website. It is the responsibility of the Principal Investigator (PI) to adapt this SOP to the specific conditions of their laboratory and submit it for review and approval by OESO Division of Biological Safety. Once approved, the PI must ensure that all primary, back-up and other personnel working with high and standard quantities of DT are trained according to the final OESO-approved SOP. It is the responsibility of the PI to ensure updates to the SOP are made in a timely manner and any major changes to the SOP are submitted to OESO Division of Biological Safety for review and approval.

## VIII. References

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5. CDC Diphtheria Antitoxin website <https://www.cdc.gov/diphtheria/dat.html>
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## Appendix I

### Duke Employee Occupational Health and Wellness Exposure/Injury Response Protocol: Diphtheria Toxin December 2018

#### RESPONSE PROTOCOL SUMMARY

##### 1. Modes of Exposure

- a. Skin puncture or injection
- b. Ingestion
- c. Contact with mucous membranes (eyes, nose, mouth)
- d. Contact with non-intact skin
- e. Exposure to aerosols
- f. Respiratory exposure from inhalation of toxin.

##### 2. Treatment

- a. First Aid:
  1. Skin Exposure/Wound: Immediately go to the sink and thoroughly wash the skin with soap and water.
  2. Splash to Eye(s), Nose or Mouth (mucous membrane): Immediately flush the area with running water for at least 15 minutes.
  3. Splash Affecting Garments: Remove garments that may have become soiled or contaminated and dispose as chemical waste.
- b. In the event of an acute injury or overt exposure, the injured employee/student should immediately inform their supervisor/PI and notify Duke Employee Occupational Health and Wellness (EOHW) via the BBF (Blood Body Fluid) Exposure Hotline at 919-684-8115 or 115 from a Duke phone. EOHW may instruct the employee/student to report to the Duke ED for medical assessment and to take a copy of their laboratory protocol document to the ED including information about the dose associated with exposure. The ED will initiate the post-exposure protocol as below.
- c. Post-Exposure Protocol after completing first aid:
  1. Inhalational exposure or accidental injection of toxin: This level of exposure may warrant deployment of antitoxin. The ED shall initiate cardiac monitoring and communicate with the CDC Emergency Operations Center at 770-488-7100 and speak with the Diphtheria Officer. EOHW will supply the most recent diphtheria toxoid IgG antibody level obtained for the exposed individual. The need for antitoxin to be administered per the CDC's protocol will be evaluated on a case by case basis.
  2. Needlestick exposure in the absence of toxin injection: The ED will review the case with the CDC Diphtheria Officer, though this would not likely be an antitoxin deployment situation.
  3. The ED should obtain a baseline EKG and cardiac enzyme levels to monitor for cardiac effects. ED will advise follow up the next business day at EOHW. Subsequent follow up will include weekly EKG, cardiac enzyme levels and a

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physical exam for three weeks to evaluate for cardiac or neurologic symptoms or complications. EOHW will consider obtaining diphtheria IgG antibody level and possible booster vaccination.

- d. Follow-up is needed in the event of **any** exposure. The employee/student is to follow up at Duke EOHW after first aid/ED treatment on the same or next business day.

## Resources

Resource	Phone	Notes
CDC - Meningitis and Vaccine Preventable Diseases Branch	770-488-7100 Emergency Operations Center 404-639-3158 MPDB office 404-639-8765 Tej Tiwari, SME for diphtheria	Controls access to diphtheria antitoxin, located in quarantine sites. May be able to have us antitoxin in 1 – 3 hours if needed.
Drusilla Burns (Deputy Director of CBER at the FDA)	301-402-3553	Recommended vaccine plus serologic surveillance of immunity
James Schmitt (Medical Director of Occ Health at the NIH)	301-496-4411	Dr. Heike Bailin, Deputy to Dr. Schmitt can also be contacted if Dr. Schmitt is not available.
John Collier (toxin expert at Harvard)	617-432-1930	
Alan Czarkowski (Medical Director of Occ Health at the CDC)	770-488-7824	