**Standard Operating Procedure**

# for Diphtheria Toxin

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| **PI Name:** **PI Signature:****Date:**  | **Building(s) and Room Number(s):** **Lab:****Animal Housing:** |
| **Designated Work Area:**  |
| **OESO Approval** **Name/Signature and Review Date:** |

1. **Hazard Identification**
	1. **Background and Risk Assessment**

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| Diphtheria toxin (DT) is a 58-kDa protein (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 (LD50) for mice has been estimated at 1.6 mg/kg by subcutaneous injection compared to an estimated human LD50 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.[**https://doi.org/10.1016/B978-012088445-2/50066-4**](https://doi.org/10.1016/B978-012088445-2/50066-4)**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). A common dose is 100 ng per injection, sometimes administered in repeated doses to achieve a cumulative effect. This dose corresponds to >=0.014 human LD50. Diphtheria toxin is commonly administered to mice in volumes ranging from 0.1 to 0.3 mL by intravenous, intraperitoneal, and intratracheal routes.**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. STAFF MUST BE CLEARED BY Employee Health (EOHW) before working with Diphtheria Toxin.For the purpose of preparation and use, user risk levels are defined according to the **Diphtheria Toxin Policy** (<https://www.safety.duke.edu/sites/default/files/Diphtheria-Toxin-Policy.pdf>). PIs and researchers must determine their risk level before making an appointment with Employee Health (EOHW) for medical review. **Read the policy for more information on what is needed.****High Risk:** 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 > = 2 microgram of toxin per sharps device **3.** Working without sharps with tubes containing > = 20 micrograms of toxin per tube **4.** Other procedures determined to be high risk by Occupational and Environmental Safety Office (OESO).**Standard Risk:** 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. |

* 1. **Preparation and Use:**

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| **Primary Dilutions for storage and use:****Note: In our lab, \_\_\_\_\_\_ and/or\_\_\_\_\_\_\_\_ are the only individuals to perform this HIGH RISK task.**In the chemical fume hood/biological safety cabinet, DT will be resuspended to a (\_\_\_\_\_\_) solution by injecting sterile water into the vial through the rubber stopper lid. This solution will be stored in aliquots of (\_\_\_\_\_) and stored (\_\_\_\_\_\_\_\_). The syringe will then be placed in the sharps waste container. Importantly, for aliquotting purpose, the rubber lid will be carefully opened and a pipet will be used to dilute, distribute, and aliquot the DT solution into Eppendorf tubes. The box for storage of DT is labeled “diphtheria toxin” and toxin hazard sign.**Subsequent Dilutions for use and Animal work:****After dosing, animals may be housed at ABSL1. Door signs and cage cards are not required for ABSL1 animal housing areas.** **Reference:** [JASN October 2005, 16 (10) 2941-2952](https://journals.lww.com/jasn/pages/articleviewer.aspx?year=2005&issue=10000&article=00020&type=Fulltext); DOI: 10.1681/ASN.2005010055(DT activity was not detected in either urine or feces) |

* 1. **Routes of Exposure and Potential Hazards:**

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| The primary risk of working with DT is **skin pricking with a needle or other sharps** which would allow entry of the toxin into the blood. Exposure may also occur via inhalation.All routes of exposure include skin, inhalation, ingestion, and injection. Target organs are lungs, kidney, bladder, heart, and nerve. Signs/symptoms of exposure: When inhaled, DT can destroy healthy tissues in the respiratory system. If the toxin gets into the bloodstream, it can cause damage to kidney, heart and nerves. The risks **are greatest when working with the toxin in powdered form and handling high concentration stock solutions**. The risks are somewhat offset by the fact that most people have been immunized against DT due the standard administration of childhood DPT vaccines. However, it should not be assumed that the vaccination alone provides complete protection to a potential DT exposure in the laboratory.DANGER: Fatal if swallowed or if inhaled!Lethal quantity per kg of body weight:Humans (≤ 100 ng i.m.) Mice (1.6 mg s.c.) |

1. **Hazard Control**
	1. **Selection and Purchasing:**

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* 1. **Engineering Controls:**

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| Within a Class II BSC, to prevent inhalation, the toxin will NOT be weighed, but will be dissolved directly in the vial as described above and stored at 4oC in its original container. The subsequent dilution (with saline before injection into animals) will also be performed in a Class II Biological Safety Cabinet (BSC) or the Chemical Fume Hood (CFH). All work with preparation of toxins (from lyophilized powder) should be conducted within the operationally effective zone of the BSC or the CFH. Each user should verify the inward airflow before initiating work. Because DT is not an infectious agent, no specific biocontainment level is appropriate. Instead, specific precautions will be implemented that are specific to working with DT. Typically, these precautions are equivalent to Biosafety Level 2. |

* 1. **Administrative and Work Practice Controls:**

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| **Recombinant DNA work that encodes for toxins must be approved by the Institutional Biosafety Committee (IBC). See** [**IBC webpage**](https://www.safety.duke.edu/biological-safety/institutional-biosafety-committee-ibc) **for more information.**DT in powdered form will never be handled in an open container.All DT solutions will be handled using disposable plasticware, which will be treated as biohazard waste after use. Specifically, no glass or pasteur pipettes or syringes will be used (other than outlined below for injections of mice). **We will limit the amount of toxin in a single syringe to ( ).** **After the mouse is injected, the empty syringe will be placed in the biohazard sharp container. We will use a separate syringe and needle for each mouse.**To avoid accidental autoinoculation, **extreme care** will be exercised when handling DT in conjunction with any injection device. * A hand-washing sink must be readily available to all locations where toxins are used.
* An emergency shower and eyewash station or eyewash/drench hose combination unit is recommended in locations where toxins are used
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* 1. **Personal Protective Equipment (PPE):**

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| When working with DT in the laboratory, personnel will wear a lab coat, gloves, safety glasses, and a mask. Within the animal facility, the standard gloves, mask, gown, bonnet, and shoe covers will be worn, along with safety glasses.  |

* 1. **Storage and Transportation:**

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| * Toxin must be placed in a secondary, shatterproof container and must be labeled with toxin name and hazard warnings at a minimum.
* When toxins are stored in the lab, containers should be sealed, legibly labeled.
* When no one is present in the lab, the lab or storage location is locked to ensure restricted access.
* Use spill trays/pads in fume hoods or BSC.
* Refrigerators and other storage containers containing biological toxins should be labeled with contact information for trained, responsible laboratory staff.
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1. **Emergencies, Spill Procedures, and Exposures/Unintended Contact**

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| *First Aid/Exposure Response*: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.
4. 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 Employee Exposure Hotline at (919)-684-8115 (115 campus landline).** 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 the exposure.
5. Prior to going to the Duke ED, a co-worker will be asked to secure toxin in locked storage, unless spilled, and secure the lab. If personnel are working alone, lab will be secured prior to going to the Duke ED. If the toxin is spilled, lab will be secured and the “*Spill Response Procedures*” below will be followed.
6. 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.

*BSC/Fume Hood Failure:*Close or cover all toxin containers. Shut down operations, close hood sash, and evacuate room. Contact your maintenance provider to repair the BSC or fume hood.* 1. Facilities Management Department: 919-684-2122 (University Buildings only)
	2. Engineering and Operations: 919-684-3232 (Medical Center Buildings only)

*Spill Response Procedures:* For spills of diluted toxin solution (inside or outside BSC):1. If personnel are exposed/injured, follow “*First Aid/Expsure Response*”.
2. Inform others of the spill and mark area as SPILL, DO NOT ENTER.
3. At minimum, safety glasses, lab coat, smock, or coveralls should be worn, along with

appropriate gloves to clean up a spill. If splashing may occur, safety goggles and a face shield must be worn in place of safety glasses. 1. Use tongs/forceps to pick up glass, if applicable and place glass in a biohazard sharps container. Do not use hands to touch broken glass.
2. Cover spill with paper towel or other disposable, absorbent material. Material will be carefully wiped up, placed into an autoclavable biohazard bag.
3. Apply freshly prepared 1:10 bleach solution to the entire area, beginning at the perimeter and working towards the center, and allowing sufficient time (**30 minutes**) to completely inactivate the toxin. Wipe area with paper towel or other disposable, absorbent material and place into the autoclave bag.
4. Autoclave at 121°C and 15 psi for 90 minutes before disposal.

For spills of toxin powder:1. If personnel are exposed/injured, follow “*First Aid/Expsure Response*”.
2. Inform others of the spill and mark area as SPILL, DO NOT ENTER.
3. Cover the spill with a 1:10 bleach moistened (wet) disposable towel.
4. Apply freshly prepared 1:10 bleach*\** solution to the entire area, beginning at the perimeter and working towards the center, and allowing sufficient time (**30 minutes**) to completely inactivate the toxin.
5. Use tongs/forceps to pick up glass, if applicable and place glass in a biohazard sharps container. Do not use hands to touch broken glass.
6. Repeat Step 4 to ensure inactivation of toxin. Apply freshly prepared 1:10 bleach*\** solution to the entire area, beginning at the perimeter and working towards the center, and allowing sufficient time (**30 minutes**) to completely inactivate the toxin.
7. Place cleanup materials in a leakproof bag for immediate disposal.

**If at any time you do not feel qualified to clean up the spill according to the instructions above, contact the OESO Spill Response team by calling 911 from a campus phone or 919-684-2444 from any phone to report the spill.** |

1. **Waste**

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| **Liquids**: *may be chemically decontaminated* (at least one method must be listed):* Name and concentration of decontamination solution: Use **1:10 bleach solution (at least 0.5% sodium hypochlorite)\***, made daily
* Time for effective decontamination: 30 minutes contact time with 1:10 bleach solution at room temperature will inactivate the toxin. After 30 minutes and while wearing face protection, pour down the drain followed by a copious amount of water.

**Solid**: All solid material to come into contact with the toxin will be disposed in biohazard bags and **autoclaved for 90 minutes at 121C before disposal**. **Sharps:** Used needles and syringes will be placed in red plastic sharps disposal containers, closed when 2/3 filled, and autoclaved for 90 minutes at 121C. **Animals:** Toxin treated animals will be disposed by DLAR’s standard method of disposal.**\***References for chemical inactivation of other protein toxins of similar molecular weight:1. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition (<https://www.cdc.gov/labs/BMBL.html>) 2. Biotechnol Prog. 2008 May-Jun;24(3):784-91. <https://doi.org/10.1021/bp070362b> Epub 2008 Apr 4.3. Chemical Inactivation of Protein Toxins on Food Contact Surfaces (J. Agric. Food Chem. 2012, 60, 26, 6627–6640 Publication Date:June 12, 2012 <https://doi.org/10.1021/jf301601v>)  |

1. **Training**

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| All personnel who work with DT will be required to: * Be trained in the theory and practice of the toxins to be used, with special emphasis on the nature of the practical hazards associated with laboratory operations. This includes how to handle transfers of liquids containing toxin, where to place waste solutions and contaminated materials or equipment, and how to decontaminate work areas after routine operations and accidental spills.
* **Read and fully adhere to this SOP when handling the toxin.**

All personnel working with this agent in animals have completed all required safety training courses and Animal Care and Use courses.Furthermore, all personnel shall:* **Be current in their diphtheria immunizations and receive clearance to work with DT from EOHW by making an appointment to discuss health considerations and risk (919-684-3136, option 2).**
* Sign this SOP documenting compliance with these requirements.
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**Standard Operating Procedure**

**for Diphtheria Toxin**

**Signature Page:**

All staff must read the final approved version of the SOP and sign this signature page to document understanding.

**“I have read and understand this SOP. I agree to fully adhere to its requirements.”**

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