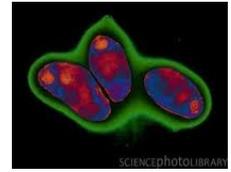


BioMaterial Fact Sheet: Diphtheria Toxin

Diphtheria toxin (DT) is a biological toxin and is secreted by the bacterium *Corynebacterium diphtheriae*. It causes damage to the host by destroying cells or disrupting normal cellular metabolism. DT inhibits protein synthesis by catalyzing ADP-ribosylation of eukaryotic aminoacyltransferase II. The lethal dose (LD₅₀) for DT is 0.1 ug/kg body weight for humans. In the laboratory setting, typical routes of exposure are from contact with the skin, through inhalation or from a needlestick (self-inoculation). Possible symptoms from an exposure include fever, headache and malaise. **The Tdap vaccination is highly recommended when working with diphtheria toxin. The vaccine is recommended to be given every 10 years. All employees working with PT and handling animals dosed with PT must be enrolled in the PT medical surveillance and report to OHS for medical clearance to participate in this protocol.**



Containment Level: Biosafety Level 2 (BSL2) criteria and animals infected with the agent should be handled and housed according to Animal Biosafety Level 2 criteria (ABSL2). *Concurrent approvals are needed from the Institutional Biosafety Committee (IBC) and the Institutional Animal Care and Use Committee (IACUC) prior to initiating work.*

Required Training: To work with *diphtheria toxin*, laboratory and animal staff must have completed the Bloodborne Pathogen (BBP) training offered by EHS as well as specialized *diphtheria toxin* training which is coordinated with the IBC Administrator. In addition, staff should receive 'hands-on' training from their laboratory supervisor or animal facility manager prior to manipulating the agent. Training should cover the hazards associated with the work, required practices and procedures and, if manipulating infected animals, proper handling of bedding, caging, and all other husbandry materials associated with the experiment.

Personal Protective Equipment (PPE): Laboratory coat (or if in animal facility, PPE per building entry requirements); gloves (double gloves recommended); face shield. *A respirator is only required for reconstitution of lyophilized powder. If working with concentrated titers and highly aerosolizing procedures contact the Biosafety Officer 301-846-5038.*

Engineering Controls: All manipulations/injections/dissections with *diphtheria toxin* or animals infected with *diphtheria toxin* must be conducted within a Biological Safety Cabinet (BSC). *No work on the open bench! A Class II Type B2 100% exhausted cabinet is mandatory for all work with the toxin in powder form. If used in animal studies, animals must be placed in ventilated caging racks maintained under negative pressure with HEPA-filtered supply and exhaust post-infection for the duration of the study. Alternate caging options may be determined and implemented on a case-by-case basis by the Animal Facility in conjunction with EHS concurrence.*

Additional Safety Practices (Laboratory):

- Two-persons are required to be present when the work is being conducted
- Wet work surface and gloves (or use antistatic gloves) prior to working with lyophilized powder to reduce static charge
- Aerosol resistant tips must be used when pipetting

Additional Safety Practices (Animal) in addition to those listed above:

- NO cage changes for 72 hours post-infection
- Only DT trained technician(s) will provide animal care for 72 hours post-infection. Other animal care staff can observe animals during this time *without* opening cages.
- 72 hours post-infections, animal cages should only be opened inside a Biological Safety Cabinet.
- Animals anesthetized for injections per PI's Animal Study Protocol (ASP)
- Only blunt tipped instruments or safety sharps (i.e. retractable, disposable scalpel) used for dissections.
- Sharps container must be kept inside engineering control where injections and dissections occur and sealed with tape
- Autoclave cages before dumping; bag cages in BSC before autoclaving
- Autoclave disposable cages, if used, prior to disposal

Disinfection (Laboratory and Animal Facility): Disinfect all work surfaces and materials *both* prior to and immediately following all work practices and procedures. (also see *Biosafety Technical Bulletin: Decontamination*)

- Surfaces: Dispatch™ or 10% (1:10) bleach solution (made daily) with a minimum of **30 minutes** contact time. Rinsing of surfaces with water is recommended after use of any chlorine-based disinfectant on metal surfaces to mitigate corrosion. **Cavicide™ and/or ethanol are not effective disinfectants for inactivating diphtheria toxin.**
- Liquid Waste: Bring liquid waste to a final concentration of 10% bleach. If aspirating into a 1L flask, flask should first be filled with ~100mL bleach and diluted down to 10% (1:10) with liquid waste. Contact time should be a **minimum of 30 minutes** prior to drain disposal (while tap water is running).
- Solid Waste: Autoclave wastes, including animals/carcasses, etc, prior to disposal, including all caging and bedding

Employee Exposure:

- Eye Exposure from splash or aerosols: Flush eyes for a *minimum* of 15 minutes in eyewash and then report to OHS immediately afterwards. Follow the NCI-Frederick Exposure Control Plan (<http://home.ncifcrf.gov/ehs/ehs.asp?id=12>) procedure for reporting occupational exposures to potentially infectious material. Dial 911 after-hours to report exposure and obtain assistance.
- Needlesticks and/or non-intact skin exposure: Wash contaminated skin for 15 minutes using a 10% povidone iodine solution (such as Betadine), a chlorhexadine scrub kit, or soap and copious amounts of water. Report to OHS immediately after scrub. If the exposure occurs after normal business hours contact the 911 emergency number. Follow NCI-Frederick Exposure Control Plan.

References:

<http://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do>
www.cdc.gov/ncidod/dbmd/diseaseinfo/diphtheria_t.htm
<http://www.tOx.in/>

Heymann, David L. Editor. Control of Communicable Diseases Manual, 18th edition. 2004. Washington, D.C. American Public Health Association. NIH DOHS