COLUMBIA UNIVERSITY

# TOXINS; BIOLOGICAL, POLICY

**Procedure: 2.11.2.**
**Version 1.1**
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| 1.0 | Preliminary Release | 09/05/2013 |
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## A. Purpose

Biological toxins are valuable research tools for studying biological processes and are also used clinically (e.g., botulinum toxin, or "botox"). These materials can be potently toxic at low concentration/volume and must be handled with utmost care according to a lab-specific standard operating procedure (SOP) and the standards set forth in Biosafety in Microbiological and Biomedical Laboratories 5th ed (BMBL). The post exposure effects of some toxins can be mitigated by prior vaccination.

## B. Applicability/scope

This policy applies to the use of all biological toxins. Toxins synthesized by recombinant means are subject to the NIH Guidelines. Deliberate formation of recombinant or synthetic nucleic acid molecules containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, and *Shigella dysenteriae* neurotoxin) require NIH/OBA and Institutional Biosafety Committee approval before initiation.

Some biological toxins are Select Agents (e.g. tetrodotoxin, conotoxin, botulinum neurotoxin). Possession of such select toxins or nucleic acids that encode functional forms of the listed toxins requires registration in the program. Some select toxins have quantity thresholds below which it is not necessary to register with the Federal Select Agents Program.

## C. Responsibilities

Principal Investigators (PI) must develop a lab-specific standard operating procedure and ensure compliance with all aspects of biosafety through submission of an Appendix E, submitted through RASCAL (https://www.rascal.columbia.edu/). RASCAL is the University's on-line research compliance system. Through RASCAL, investigators can create and submit for regulatory approval most research protocols. The Appendix E must include emergency procedures in the event of environmental, facility contamination or personnel exposure, and indicate antidotes if available. If biological toxins are administered to animals, the Appendix E must be attached to the IACUC protocol. If not, it should be submitted as an "in vitro" protocol. If biological toxins are synthesized by recombinant means, *in vitro* or *in vivo*, an Appendix A must be submitted. All researchers that handle biological toxins must be PI-approved, trained, and have received vaccination, if available (or declined by signing a waiver of vaccination; example shown in Appendix 1). The PI must declare any proposed Select Agent toxin work when creating a proposal in the RASCAL proposal tracking application, and then maintain inventory below the threshold required for Select Agent Program registration. Should investigators need to maintain select toxin inventory above the threshold they must contact EH&S to facilitate the registration process with the Federal Select Agents Program. EH&S is also available to consult on all aspects of this policy.

## D. Definitions

Select Agents – Select agents or toxins are bio-agents which have been declared by the US Department of Health and Human Services or by the US Department of Agriculture to have the potential to pose a severe threat to public health and safety. Many activities with Select Agents require registration with the Federal Select Agent Program and close regulatory oversight of related activities.

**E. Procedures**

EH&S must be notified prior to the initiation of any work with biological toxins by submission of an Appendix E. Depending on the type of toxin and quantities in use, Environmental Health and Safety (EH&S) staff may work with the PI to develop a detailed lab-specific SOP that identifies engineering controls, PPE, lab procedures, disposal/disinfection practices, medical surveillance, and exposure/spill response (example for Diphtheria toxin shown in Appendix 2). This document must be maintained in the laboratory as a hard copy. Toxin users must maintain documented written approval from the PI. All exposures to biological toxins must receive prompt medical evaluation. EH&S must be notified of accidents or exposures.

**F. Emergency contacts**

N/A

**G. Medical Surveillance**

Where vaccination exists, and is commercially available (e.g. Diphtheria, Pertussis), this should be made available at no cost to employees and in some cases may need to be paid for by the PI. Workforce Health and Safety, or Student Health Services can prescribe and administer vaccine. Those declining the offer of vaccination must sign a declination of vaccination form, which also includes a waiver of claims (example shown in Appendix 1). Print & Go post exposure guidance sheets are available for diphtheria and pertussis toxins http://ehs.columbia.edu/PrintAndGo.html

**H. Recordkeeping**

Hard copies of lab-specific SOPs, approved use log, lab-specific training, approved personnel rosters and vaccination declinations are maintained by the PI for three years following termination of the protocol.

**I. Appendices**

Appendix 1 – Example declination of vaccination form for Diphtheria toxin
Appendix 2 – Example detailed lab-specific SOP for Diphtheria toxin

**J. Forms**

None

**K. References**

National Select Agent Registry http://www.selectagents.gov/
Possession, Use and Transfer of Select Agents and Toxins
http://www.ehs.columbia.edu/SelectAgents.html

**Appendix 1**

## DECLINATION OF DIPHTHERIA VACCINATION – WAIVER & RELEASE OF ALL CLAIMS

### Acknowledgement of Risk of Refusal to Receive Vaccination

I have been provided training on the safe handling of diphtheria toxin, including the benefits of vaccination. I understand that due to occupational exposure to diphtheria toxin I may be at increased risk of morbidity and mortality if I have not been vaccinated in the last 10 years with Tdap (Tetanus-Diphtheria-Pertussis) or Td (Tetanus-Diphtheria) vaccine. I have been given the opportunity to be vaccinated with the Td vaccine at no charge to me; however, I decline vaccination at this time. I understand that by declining this vaccine, I may be at increased risk of morbidity and mortality. If, in the future I continue to have occupational exposure to diphtheria toxin and I want to be vaccinated with Tdap or Td vaccine, I can receive the vaccination at no charge to me.

### Waiver of Claims

*Please understand that in refusing this vaccination and signing this document you will be waiving and releasing on behalf of yourself, your spouse and your dependents all claims as a result of disease, death or for injuries, including but not limited to the aggravation of any pre-existing ailment or condition; disability and disfigurement; pain and suffering; medical care, treatment and services, lost earnings, profits and salaries; lost earning capacity; the reasonable expense of necessary help in the home; as well as any property damage that might be sustained arising directly or indirectly out of your refusal to receive the vaccinations.*

I do hereby fully release and hold harmless The Trustees of Columbia University in the City of New York, as well as any and all of its officers, agents, servants, employees, independent contractors and volunteers from any all claims as a result of disease, death or from injuries, including but not limited to the aggravation of any pre-existing ailment or condition; disability and disfigurement, pain and suffering; medical care, treatment and services; lost earnings, profits and salaries, lost earning capacity; the reasonable expense of necessary help in the home; as any and all property damage I, my spouse or my dependents might sustain arising directly or indirectly out of my refusal to participate in the above-captioned Diphtheria Vaccination Program.

I have read and fully understand the Waiver & Release of All Claims

_____
Participant's Signature

_____
Print Name

_____
Date

## Appendix 2

# Safe working procedures for Diphtheria Toxin (DT) at Columbia

**Background**

Diphtheria toxin (DT) is a potent lethal toxin in humans. The minimum lethal dose is 100 ng/kg (6-10 ug). Diphtheria toxin is a single protein with two parts: one that allows entry into host cells and the other prevents the host cell from making proteins. The toxin binds to a cell-surface receptor to gain entry into the cell. Inside the cell, the protein prevents the cell from making new proteins.

In 1924, Ramon demonstrated the conversion of diphtheria toxin to its nontoxic, but antigenic, equivalent (toxoid) by treating with formaldehyde. He provided humanity with one of the safest and surest vaccines of all time, the diphtheria toxoid.

In 1951, Freeman made the remarkable discovery that pathogenic (toxigenic) strains of *C. diphtheriae* are lysogenic, (i.e., are infected by a temperate Beta phage), while non-lysogenized strains are avirulent. Subsequently, it was shown that the gene for toxin production is located on the DNA of the Beta phage.

In the early 1960s, Pappenheimer and his group at Harvard conducted experiments on the mechanism of action of the diphtheria toxin. They studied the effects of the toxin in HeLa cell cultures and in cell-free systems, and concluded that the toxin inhibited protein synthesis by blocking the transfer of amino acids from tRNA to the growing polypeptide chain on the ribosome. They found that this action of the toxin could be neutralized by prior treatment with diphtheria antitoxin. Subsequently, the exact mechanism of action of the toxin was shown, and the toxin has become a classic model of an ADP-ribosylating bacterial exotoxin.

**Engineering controls:**

Dilution and aliquoting of DT will be performed in the fume hood in room XXXX of the XXXX building. Injections will be performed in fume hoods in the XXXX vivarium (room XXXX).

**PPE:**

Disposal gown, double nitrile gloves and eye protection.

**Lab procedures:**

*Reconstitution:* This is the most hazard-prone part of the procedure since the total amounts of toxin being handled could represent a lethal amount in humans. DT will be obtained from List Biological Laboratories as lyophilized powder (500 ug/vial) and stored in room XXXX. It will be diluted in water (2 ug/uL) and stored in 50 ul aliquots at -20 degrees in the chemical freezer in XXXX room XXXX. To prevent DT powder aerosolization, lyophilized containers should be not opened. Water should be added to the lyophilized stock through the rubber septum using a syringe needle. See below for safe sharps practices. These stocks are further diluted to a working solution of 2ug/ml.

*Animal work:*

**I/P injections**
Mice will be injected with approximately 0.4-40ng/kg DT (~10-1000pg per mouse). Each mouse, approximately 100 in total, will be injected approximately 1-8 times. Injections will be performed in a fume hood in the XXXX mouse vivarium.

**I/C injections**
Mice will be injected with 1uL of 100pg/uL DT bilaterally via intracranial injections. Each mouse will be injected once. Injections will be performed in the fume hood in the XXXX mouse vivarium.

Research indicates that DT activity is not present in urine or feces of rats that received dose of up to 50 ug/kg (Wharram et al., 2005, J Am Soc Nephrol). Since DT activity is not present in excreta, no special procedures will be used in handling mouse bedding and cages. Carcasses will be placed in regulated medical waste bags, which will be sealed and placed into the carcass freezer on the XXXX floor XXXX building (depending on where euthanasia occurs). Mice will not be sacrificed until at least 1 month after DT injection; at this time point the presence of active DT in the carcass is unlikely.

*Work with needles:* Needles should not be recapped. Needles and syringes are used once only.

**Disposal:**

*Sharps:* Sharps include needles and pipette tips. Sharps require chemical decontamination prior to disposal. 10% bleach (made daily) should be drawn up into used pipette tips and syringes. The pipette tips or the whole syringe should be placed into a small sharps bin in the fume hood, labeled "Diphtheria toxin disposal only". This bin should be stored where the work is being performed. Small sharps bins should be disposed of intact and can be added to a larger reusable sharps bin.

*Solid waste:* includes tubes, absorbent paper towels. These can be disposed into the regulated medical waste (red bag waste).

*Liquid waste:* Small amounts of liquid in capped tubes can be disposed in the regulated medical waste. Larger liquid stocks should be decontaminated before disposal by mixing with bleach to give a final concentration of 10%. This can then be disposed into the sewer drain after 30 min. contact time.

*Bedding and carcasses:* No special provisions required.

**Principal Investigator (PI) responsibilities:**

The PI will ensure that all lab personnel are trained in the use of safe laboratory procedures to prevent accidental exposure before assignment to any laboratory where diphtheria toxin is used.

The PI will carefully explain the necessity of immunization with diphtheria toxoid. The PI will ensure that all laboratory workers are either fully immunized against diphtheria.

The PI may request assistance from EH&S in providing information about safe laboratory procedures and the necessity of immunization with diphtheria toxoid.

The PI must ensure that all researchers who will be working with diphtheria toxin have read this protocol. The PI will also ensure that the protocol will be reviewed on a yearly basis by all laboratory workers.

The PI will maintain a log that documents protocol review, training, and immunization status of approved DT users.

**Medical surveillance:**

Documented Tdap vaccination or Td booster within prior 10 years (or written declination) is required prior to working with Diphtheria toxin (DT).

**Exposures:**

All DT exposures should receive immediate medical evaluation at Student Health Services, Workforce Health and Safety, or the NYP-ER for after-hours exposures. EH&S, the Principal Investigator, and Workforce Health and Safety should be notified immediately. An estimate of the amount of DT exposure (milligram, microgram, nanogram) should be made. Take a copy of this protocol to the medical provider.

SUBCUTANEOUS EXPOSURE
If injected, remove gloves. Wash with soap for 10 min. and express the wound under running water.

ORAL EXPOSURE
If swallowed, wash out mouth with water, provided person is conscious.

INHALATION EXPOSURE
If inhaled, remove to fresh air. If breathing becomes difficult call 911.

DERMAL EXPOSURE
In case of skin contact, wash the skin thoroughly with soap and water for 10 min. Rinse with copious amounts of water. Remove contaminated clothing and shoes.

EYE EXPOSURE
In case of contact with eyes, flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers.

**Physician information:**

*Symptoms:* Methods for identifying exposure include fever, headache, and malaise. Skin contact causes rash.

There have been reports of rapid onset local pain after percutaneous exposure to toxin, and such an occurrence would indicate a significant exposure. Onset of symptoms following significant toxin exposure would typically be delayed by days to weeks, and are due to the inhibition of protein synthesis. The physician shall assess the severity of the exposure, and take appropriate actions to include consultation with CDC. Treatment with immune globulin may be considered in the absence of symptoms in case of an especially severe or large exposure.

*Standard treatment:* Td booster is the typical treatment for exposures to DT.

*Antitoxin therapy:* In case of unprotected, accidental exposure to toxin, or exposure to toxin that exceeds the protective capacity of neutralizing antibodies in immunized individuals, treatment with hyperimmune antiserum (antitoxin) has been shown to reduce mortality from 7 to 2.5 percent. Antibodies only neutralize toxin before its entry into cells, so rapid treatment is essential. Because the antitoxin is produced in horses, up to 10 percent of treated individuals may develop serum sickness. Contraindications/Precautions in the use of equine immune globulin include a history of prior exposure to horse serum, prior history of serum sickness, or a history of asthma or hay fever, especially when near horses. Although diphtheria antitoxin is no longer licensed by the FDA in the United States, a European-licensed product is available from the National Immunization Program of the CDC as part of an investigational new drug (IND) protocol by calling **404-639-8200**. CDC Emergency Operations Center can also be contacted after hours at **(770) 488-7100**. Typically it is only released by CDC to treat Corynebacterium diphtheria infections. It has not been titrated experimentally based on the quantity of toxin exposure or prior immunization status. Release of antitoxin will be made by CDC on a case by case basis. The physician treating the exposed patient should call CDC to discuss the case. If a decision is made to release the antitoxin, the IRB at CDC has preauthorized release to expedite treatment. There will be paperwork that needs to be completed for the FDA post treatment. The CDC website for diphtheria toxin requests and forms is:

http://www.cdc.gov/vaccines/vpd-vac/diphtheria/dat/dat-main.htm

This web page is shown in Appx.2. The IND protocol is Appx. 3 and the consent form is Appx. 4.

The emergency room should draw at least ten milliliters of serum and hold it for possible toxin assay. This must be done before any treatment with antitoxin.

Any patient seen and released should be given information about the potential for delayed onset of symptoms/toxicity. Any symptoms would be reason for emergent reevaluation.

Diphtheria toxin catalyzes the ADP-ribosylation (and inactivation) of the elongation factor eEF-2 that is essential to protein synthesis. This is reversible by giving high doses of nicotinamide. Nicotinamide is a water-soluble vitamin and is part of the vitamin B group. However this is an experimental treatment with no clinical trials having been performed.

**Environmental or facility contamination:**

SMALL SPILLS: Allow aerosols to settle; wearing protective clothing, gently cover spill with absorbent paper towel and apply 10% sodium hypochlorite, starting at perimeter and working towards the center; allow sufficient contact time (30 min) before clean up

LARGE SPILLS: Leave the area and secure the lab to prevent entry of other personnel, and possible secondary exposures. Call EH&S and Public Safety.