



## Biological Toxins

Biological toxins are toxic substances that can be produced by bacteria, fungi, protozoa, insects, animals, or plants and are classified separately from chemical toxins. They are nonreplicative, noninfectious materials but can be extremely hazardous, even in minute quantities.

This document provides guidance safety precautions for work with biological toxins and policies and regulations that may apply to research with biological toxins, including review by the Institutional Biosafety Committee (IBC), Federal Select Agent and Export Compliance regulations.

### Potential Health Hazards

The toxicity and health hazards of biological toxins vary greatly and toxins may be cytotoxic, neurotoxic, hemolytic, or cause necrosis. Of primary concern are acute biological toxins.

### Modes of Transmission

Biological toxins may be transmitted via surface contact with contaminated object(s) and subsequently spread to mucus membranes (eyes, nose, and mouth) and/or to open sores on skin. Some biological toxins can be absorbed through intact skin, especially if solubilized in substances such as dimethyl sulfoxide (DMSO). Accidental needle-stick is a mode of transmission within research laboratories. Accidental ingestion of contaminated materials and inhalation are other routes of transmission.

### Stability

Biological toxins are fairly stable in undiluted forms but usually do not persist for long periods of time in the environment. Some toxins, such as Ricin are very stable and can persist in the environment.

### Laboratory Practices

**Biosafety Level 2** practices and facilities shall be used for activities involving biological toxins.

- Biohazard signs and labels must be displayed in areas and on equipment where biological toxins are used and stored. This includes, but is not limited to, laboratory entrance doors, biological safety cabinets, chemical fume hoods, refrigerators, and freezers.
- Use a biological safety cabinet (BSC) (a.k.a. tissue culture hood) or a chemical fume hood for resuspension of biological toxins or manipulations of stock solutions of toxins that can generate aerosols, such as pipetting, harvesting, infecting cells, filling tubes/containers, and opening sealed centrifuge canisters.

- Some toxins come in sealed glass ampoules that must be “snapped” to have access to the powder within for resuspension. Use great care in cracking the ampoule to avoid an accidental cut.
- Some toxins arrive in rubber septum sealed vials. If using a needle and syringe to plunge through the septum to resuspend, then use a hands-free device to stabilize the vial to avoid an accidental needle stick. Use great care.
- Whenever possible, use needle-free techniques to resuspend biological toxins.
- If you must weigh a quantity of powder-form toxin, then the scale must be located in a certified chemical fume hood.
- See “Inactivation and Disposal” below for additional information.

## Personal Protective Equipment

Work with biological toxins shall be conducted using the following PPE within a BSL2 facility:

- Disposable gloves – consider the use of double gloves for enhanced protection. Ensure your gloves are compatible with any solvent your toxin may be dissolved in.
- Lab coat or back-closing disposable gown
- Eye protection (safety glasses or goggles) is recommended, but not required when working within a biosafety cabinet or a chemical fume hood.

## Precautions When Using Animals

All work with biological toxins in animals must be reviewed by the OHSU Institutional Animal Care and Use Committee (IACUC). See the IACUC webpage for more information:

<http://www.ohsu.edu/xd/research/about/integrity/iacuc/index.cfm>

The Animal Biosafety Level (ABSL) designation will be determined by the IBC or Biosafety Officer during project review. ABSL-1 handling and containment is usually sufficient for *in vivo* projects with biological toxins. If your work requires ABSL-2 containment and work practices, then the Department of Comparative Medicine (DCM) will provide training to ensure safety requirements are met. The PI must contact DCM at least two weeks in advance to reserve ABSL2 lab space.

## Inactivation and Disposal

Toxin stability varies considerably outside of physiological conditions depending upon the temperature, pH, ionic strength, and other characteristics. Inactivation is not always a linear function of heating time, and some protein toxins possess a capacity to re-fold, and partially reverse inactivation caused by heating. In addition, the conditions for denaturing toxins in aqueous solutions are not necessarily applicable for inactivating dry, powdered toxin preparations.

Recommended parameters for chemical or heat inactivation of some common biological toxins can be found in Section 8.5 of the [OHSU Biosafety Manual](#). Additional information may also be found in [Section VIII-G](#) of the 5<sup>th</sup> Edition of the BMBL.

Materials that are potentially contaminated with toxins shall be disposed of as biohazardous waste and sharps must be disposed of in a red sharps container.

## Employee Exposure

All personnel working with biological toxins or accessing a toxin laboratory should be familiar with the signs and symptoms of toxin exposure. Researchers working with a toxin should be vaccinated if a vaccine is available for the biological toxin (e.g. diphtheria toxin, tetanus toxin). Antitoxins are available for some biological toxins and immediate medical “first-aid” interventions may help prevent or lessen the severity of the reaction.

If you know or suspect a biological toxin exposure...

- **Irrigate the site of exposure**
  - If exposure was by needle stick or other route which breaks the skin, wash with soap and water for 5-15 minutes and cover with a bandage.
  - If exposure was by splash to eyes or mucus membranes, irrigate thoroughly for 15 minutes at an appropriate eye wash station.
  
- **Report to your Laboratory Supervisor and Biosafety Officer IMMEDIATELY. Seek medical attention as outlined in your laboratory specific safety manual.**

## Spill and Disposal Procedures

Refer to the [OHSU BSL2 Manual Template](#) spill response cue cards.

## Questions/Concerns for the Biosafety Officer?

- **Central Campus: 503-494-0655 or 503-494-2580**
- **West Campus: 503-690-5312 or 503-690-5368**

## IBC Registration

Research at OHSU involving biological toxins that are on the Select Agent list or have an LD50 in vertebrates less than or equal to 100 ng/kg must be reviewed and approved by the Institutional Biosafety Committee (IBC) prior to start of work.

For work with the biological toxin; complete an [Infectious Agent/Toxin Questionnaire \(IAQ\)](#).

Work involving recombinant or synthetic DNA encoding the active subunit(s) of biological toxins with an LD50 of less than 100 ug/kg in vertebrates in cells, organisms, or viruses must also be documented on a [Recombinant DNA Research Questionnaire \(RDRQ\)](#), approved by the IBC, and may require additional review by the National Institutes of Health Office of Biotechnology Activities (NIH-OBA) as indicated in [Section III-B-1](#) and [Appendix F](#).

## Select Agent Toxins

Some biological toxins are classified by the Federal Government as [Select Agents](#) due to their potential to pose a severe threat to public health and safety. Possession, use, and transfer of these biological toxins is highly regulated.

The toxins do not require registration with the federal government if the amount under the control of a principal investigator, treating physician or veterinarian does not exceed at any time the [permissible toxin amounts](#), which are also indicated in the table at the end of this fact sheet.

**The laboratory must maintain an inventory of the amount of the Select Agent toxin present in the laboratory at any given time.** This inventory should document the number of vials containing toxin, amount in each, amount remaining (if applicable) after each use, and how the toxin was inactivated when no longer needed for experiments.

**The Federal Select Agent Program has specified that researchers must also exhibit [due diligence](#) regarding any transfer of a select agent toxin** in order to block someone attempting to stockpile these toxins. Researchers shall document who the recipient is and that this individual has legitimate purpose to handle and use such toxins. Information pertinent to the person requesting and using the toxins such as the individual's name, institution name, address, telephone number, and e-mail address should be documented. Documentation of a legitimate research purpose may be done by—

- requiring the recipient to complete documentation stating their intended use of the toxins
- the OHSU researcher can document their knowledge of the recipient's legitimate need for the toxins
- any researcher wanting to transfer a Select Agent toxin must first contact the biosafety officer for assistance in this transfer.

## Transport Requirements

Materials must be appropriately contained, sealed, and labeled for transport within

Shipping infectious substances, toxins, diagnostic specimens, and/or dry ice off-campus requires training and certification. Individuals must take the Dangerous Goods Shipping Training available on [Big Brain](#). Please consult a Biosafety Officer for any regulations that must be met if you are shipping biological toxins.

Some biological toxins and genetic elements encoding these toxins are on the Commerce Control list and are subject to Export Control regulations. A list of biological toxins on the Commerce Control list is noted in the table at the end of this fact sheet. Please contact the Export Compliance Officer or visit the [OHSU Export Controls website](#) if you have questions.

## Table of Biological Toxins

This table focuses on biological toxins that are subject to federal regulations and is by no means comprehensive of all biological toxins. The table indicates the LD50 for some acute biological toxins and the permissible amounts of Select Agent toxins.

Toxin	LD50 (ug/kg)	Requires IBC Review	Select Agent	Export Compliance
Abrin	0.7	Y	Y (100 mg)	Y
Aerolysin	7			
Aflatoxins				Y
$\beta$ -bungarotoxin	14			
Botulinum toxins	0.0004 to 0.0025	Y	Y (0.5 mg)	Y
Caeruleotoxin	53			
Cereolysin	40 to 80			
Cholera toxin	250			Y
Clostridium difficile	0.5 to 220			
Clostridium perfringens	0.1 to 1500	Y		Y
Conotoxins	12 to 30	Y	$\alpha$ -conotoxins only, (100 mg)	Y
Crotoxin	12 to 30			
Diacetoxyscripenol toxin		Y	Y (1000 mg)	Y
Diphtheria toxin	0.1	Y		
HT-2 toxin	5 to 10			Y
Listeria listeriolysin or hemolysin	3 to 10			
Leucocidin	50			
Microcystin (Cyanginosin)				Y
Modeccin toxin	1 to 10			Y
Nematocyst toxins	33 to 70			
Notexin	25			
Pertussis toxin	15			
Pneumolysin	1.5			
Pseudomonas aeruginosa exotoxin A	3			
Ricin	2.7	Y	Y (100 mg)	Y
Saxitoxin	8	Y	Y (100 mg)	Y
Shiga Toxin	20			Y
Shigella dysenteriae neurotoxin	1.3			
Staphylococcal aureus toxins	2 to 25	Y	Subtypes A -E (5 mg)	Y
Streptolysin S	25			
Taipoxin	2			
Tetanus toxin	0.001	Y		
T-2 toxin	5 to 10	Y	Y (1000 mg)	Y
Tetrodotoxin	8	Y	Y (100 mg)	Y
Verotoxin				Y
Viscum Album lectin 1 (Vixumin)	2.4 to 80			Y
Volkensin toxin	1.4			Y
Yersinia pestis murine toxin	10			