Vanderbilt University Institutional Biosafety Committee (IBC) Policy:
BIOSAFETY BEST PRACTICES FOR RESEARCH USE OF ACUTELY HAZARDOUS BIOLOGICAL TOXINS

This document applies to the research use of biological toxins that are:

1. received in a premeasured quantity packaged in a septum vial that will not be opened prior to reconstitution with a vial adapter*; and
2. meets one or more of the following criteria:
    - toxin presents a life-threatening or severe irreversible health effect risk in a single exposure incident scenario (i.e., acutely toxic);
    - toxin is included on the CDC/APHIS Select Agent List.

Biological toxin work that meets either criterion above may require registration with, and approval by, the Institutional Biosafety Committee (IBC) as outlined below. Common examples of toxin use that meet the criteria above include:

- tetrodotoxin used in cell culture or on animal protocols,
- staphylococcal enterotoxin B (SEB) used for cell culture, and
- administration of diphtheria toxin on animal protocols.

** Please note that all efforts should be made to order toxins packaged in septum vials that can be accessed using a vial adapter. If a toxin meeting the criteria above is received in a premeasured quantity in a screw top vial, this policy may be applied provided that a justification/rationale is documented in procedures and, the only manipulation performed is addition of diluent to the vial following safety practices outlined in the "Exposure Hazards" section. If work with acutely toxic materials is planned that requires manipulation of dry toxin, that work does not fall under this policy. Consult VU EHS Chemical Safety in that circumstance.*

Institutional Biosafety Committee Review & Approval for Possession and Use of Toxins

To gain approval for this activity, the following 4 actions must be completed.

1. The PI must complete and submit a Vanderbilt Biological Materials Registration (or amend an existing registration).

2. The PI must identify and assign the role of toxin mentor to a senior lab member who will be responsible for toxin user training and qualification, and surveillance of toxin inventory and activity records. This individual should be fully versed in the toxin use policy, the lab's toxin SOPs, and be empowered by the PI to ensure compliance with toxin use policy and procedures.

3. The PI (in conjunction with the toxin mentor) must prepare and maintain a Toxin Safety Plan in the lab that includes these 5 components:
    1. A copy of this policy document;
    2. Manufacturer's safety data sheets (SDS) for the toxin(s) to be used;
    3. Inventory for each toxin which will allow the end user to quickly and clearly communicate how much toxin is on hand in storage. Undiluted septum vials, diluted stocks and aliquots should be documented.
    4. Standard operating procedures (SOPs) that outline all technical and safety steps involving toxin handling (see Attachment 1);

5. Training records for all personnel authorized to handle the toxins(s) (see Attachment 2).

4. VU Biosafety will meet initially with the toxin mentor and lab personnel who plan to use toxins to review the proposed procedures and provide guidance on procedural refinements and any toxin use items in need of further consideration or action. Biosafety will share the lab's toxin safety plan status with the IBC and request approval on the lab's behalf.

To maintain IBC approval for research activities involving acutely hazardous biological toxins, the PI must ensure that they respond to activity surveys received from VU Biosafety and complete actions that apply to any activity changes in a timely manner.

If the lab's activities involving toxin use are paused for more than one year, and no plans for toxin use are included in current grant proposals, the PI should coordinate with VU Biosafety to transfer or dispose of remaining toxin and toxin aliquots. This action will relieve unnecessary regulatory burden and liability for all parties.

Exposure Hazards Associated with Toxins

Manipulation of dry toxin product can be a significant exposure risk through accidental inhalation and through dispersal of toxin residues to the surrounding environmental surfaces. For these reasons, use a premeasured product that will permit the diluent to be added without the need to open the container and manipulate dry toxin. The most common configuration for this is a septum vial that can be fitted with a vial adapter (VU Biosafety can provide upon request). This configuration should be purchased whenever possible.

If premeasured toxin product must be received in a screw top vial, the potential for dispersal of toxin residue exists and must be addressed. Specific <u>additional</u> containment procedures include the following:

- Wear anti-static nitrile gloves and anti-static sleeve protectors. (Note: latex gloves generate static electricity which contributes to dispersal.)
- Place a "drop cloth" on the BSC or fume hood work surface, then lightly dampen it with a spray of freshly prepared 1:5 bleach solution to remove any dust or lint fibers in the absorbent material that may generate static electricity.
- Unscrew the vial cap using gauze prewetted with water to prevent the dispersal of toxin residues caused by static.

It is also advised that the primary container be one that is non-breakable if possible (i.e., safety coated glass). Vials should be maintained in a closed secondary container that will not allow escape of the product if it is dropped. To achieve this, use a rigid plastic container with a screw cap or latchable lid. To further stabilize vials, place them in a rack or Styrofoam tray inside the secondary container. By using secondary containers at all times, the potential for a release of dry or liquid toxin from the primary container is greatly decreased.

The other significant exposure risk scenario is an accidental injection or cut while transferring or administering the toxin with a sharp device. The use of sharps on procedures should be minimized as much as possible and those handling these devices need to follow sound safety practices to protect themselves and others who share the lab space from exposure to the toxin. Sharps handling procedures should be carried out in accordance with the document entitled: *Using Sharps Safely in Lab Research Applications*. Please note that for toxin applications, the additional following provisions apply:

- Use only syringes with luer-lock or integrated needles.

- Use vial adapters whenever possible to eliminate the need to use a needle to add diluents into a septum vial.
- If you must introduce a needle through a septum because a vial adapter is not available for the vial configuration, assure that the vial is secured with a device that allows the non-dominant hand to be outside of the "strike zone" of the needle. Either secure the vial in a rack or use a clamp to hold the vial instead of holding it directly by hand during needle introduction and removal.
- Use a safety engineered device (i.e., one that has a mechanism to enclose the sharp end/edge after use) if one is available and feasible to use on the procedures.
- When administering toxins to animals, be well-trained in physical restraint techniques before attempting to administer any hazardous materials with a sharp device.

Personal Protective Equipment

Personal protective equipment worn for toxin manipulations should prevent the potential for toxin contamination to contact your personal clothing or exposed skin. A wraparound fluid-resistant disposable gown with gathered cuffs is a good option for protecting your clothing when worn properly. Fluid-resistant disposable gloves rated for protection against the diluent should be used. Double-gloving is strongly recommended if it does not hinder the wearer's ability to carry out the procedures at hand safely and efficiently. Safety glasses are necessary for procedures that must be carried out outside of a fume hood or BSC (i.e., animal challenges that must be performed on the open bench due to method of administration).

Gowns and gloves are single use and must be disposed of as biohazardous waste (or toxin waste if applicable). Safety glasses must be washed after removal and before storage. While personal clothing is not rated as "personal protective equipment", it is strongly advised that persons handling hazardous agents do not have unprotected exposed skin when working in that environment. Long pants and footwear that fully covers the foot should be worn to minimize the potential for accidental direct skin contact with the toxin.

Toxin Inactivation and Waste

Because each toxin is biochemically different, there is no universal method of inactivation for toxins. While VU Biosafety can provide guidance in that area, it is critical that researchers who are using toxins in their studies become knowledgeable in the methods of inactivation for their particular toxin. When preparing decontamination information for standard operating procedures, list the applicable reference. Additional considerations specifically related to toxin inactivation include the following:

1. Drape working areas where toxins are to be manipulated with absorbent towels that can be easily collected and discarded following procedures. If working with concentrated stocks, fully decontaminate the work area after removal of the drapes. If performing animal procedures with diluted product to be administered, clean and disinfect the area after removal of the drapes using facility disinfectant.
2. Solid waste items (i.e., gloves, waste vials, bench paper, etc.) should not be soaked/submerged in a liquid decontamination solution.
    - If the toxin can be inactivated by autoclave treatment, solid waste should be collected in an autoclavable bag and sterilized via autoclave before it leaves the lab for final treatment and disposal.
    - If the toxin cannot be inactivated by autoclave, the waste will be collected as solid toxic waste in accordance with the VU EHS Hazardous Chemical Waste Guide. (In short, waste will be collected in a labeled, lined bucket with a lid and hazardous waste tag.)
3. Sharps contaminated with a toxin may be disposed of in biohazardous sharps containers.

4. Liquid waste containing a toxin that can be inactivated with bleach may be disposed of via the lab sink, similar to other bleach-treated biological liquids. If the toxin is not inactivated by bleach, the need for collection as liquid hazardous waste may apply. VU Biosafety and Chemical Safety will assist with this determination.

Spills & Exposures

Preparation for spills and exposures is necessary before any toxin handling takes place. Because there is no universal decontaminant, spill procedures will need to be tailored for the toxin lab's needs. In general, the basic steps for responding to a spill of liquids containing toxins are outlined below:

1. **Isolate the area.** (This should already be done in the case of toxin use. Only those who are authorized for toxin work should be permitted into lab areas where toxin use is underway.)
2. **Remove the breached container.** If breached container is glass, remove glass pieces using tongs or disposable broom/dustpan. Place glass in sharps container for disposal. If container is not glass, place it in a plastic bag for treatment and disposal or appropriate secondary container.
3. **Treat, absorb and remove the spill contamination.** Cover spill with decontaminant-saturated towel and allow contact with spill for several minutes. Absorb and remove spill contamination. (Use tongs or other tools to minimize direct handling of spill materials if feasible.) Place absorbed spill materials and associated wastes in plastic bag.
4. **Decontaminate all impacted surfaces.** Apply decontaminant to all surfaces impacted by the spill (including those in the "splash zone"); wait the prescribed contact time before removing decontaminant residues.
   NOTE: Use care to limit contact with contaminated surfaces when removing PPE. Place all used spill response materials (including mechanical tools and disposable PPE) in the plastic bag for final treatment and disposal. Contact VU Biosafety for assistance with storage and disposal of spill waste.

As part of their assessment of toxin activities, Occupational Health may prescribe specific actions to take if a toxin exposure incident (i.e., needlestick, contact with unprotected skin or eyes) occurs. Generally speaking, in the event that an ***exposure incident*** occurs, the exposed person should take the following actions immediately:

1. Proceed to the closest sink/eyewash. Remove impacted PPE and flush the exposure site.
2. If the exposure involved broken or compromised skin, use soap and water to thoroughly cleanse the wound. (Do not use bleach or other harsh chemicals that can degrade tissues.)
3. Flush/cleanse the exposure site for 15 minutes.
4. Cover the wound with a bandage (if applicable).
5. Report immediately to VUMC Adult Emergency Department with a copy of the protocol (to identify the potential exposure dose). The Emergency Department will consult with Occupational Health.
6. Notify the LAB SUPERVISOR and Biosafety Officer (BSO) at VUBiosafety@vanderbilt.edu as soon as possible once medical follow-up actions have been initiated.

Restricted Access to Toxins

Many biological toxins are regulated under the CDC select agent rules which means that only a minimal amount of toxin can be maintained by a Principal Investigator (PI) (see table below). Additionally, the toxin must be secured and accounted for at all times. The toxin cannot be transferred to another PI without notification of, and approval by, VU Biosafety. In all circumstances, personnel who have exposure potential to biological toxins that pose an acute high risk exposure hazard as described above need to be on record with Occupational Health for timely and effective medical response in the event of an exposure. Because of these

two factors, toxins need to be maintained in a secure storage device (i.e., lock box in a locked storage unit or lock box in a storage unit inside a locked lab) that can only be accessed by personnel authorized to work with the toxin.

When toxins are in use, the area where the work is being conducted should be posted as "toxin use in progress-authorized personnel only" or equivalent and VU Biosafety can provide this signage. Under routine circumstances, no one should enter the area where this work is being conducted aside from personnel authorized to work with the toxin.

SELECT AGENT-LISTED TOXINS

Toxin Name	Per PI Limit*
Abrin	1000 mg
Botulinum neurotoxins	1 mg
Short, paralytic alpha conotoxins	200mg
Diacetoxyscirpenol (DAS)	10,000 mg
Ricin	1000 mg
Saxitoxin	500 mg
Staphylococcal Enterotoxins (Subtypes A, B, C, D, and E)	100 mg
T-2 toxin	10,000 mg
Tetrodotoxin	500 mg

*This is the maximum amount that a PI may possess without becoming registered with the CDC for possession of a select agent.

Due diligence requirements and responsibilities apply to ALL PI's possessing a select agent-listed toxin regardless of quantity.

Please note: Beginning May 6, 2025, any research use of the select agent-listed toxins above will be subject to dual use research of concern (DURC) review and institutional documentation of a completed DURC review may be required during grant review by federal funding agencies. Please contact the Research Integrity and Compliance team or VU Biosafety for more information.

Training

Work with toxins of biological origin should be carried out in accordance with Appendix I of the CDC/NIH "Biosafety in Microbiological and Biomedical Laboratories", 6th edition. Under this standard, BSL-2 containment applies. Therefore, all personnel working with toxins in a scenario covered by this Best Practices document should complete the following before handling toxins:

1. Complete institutional training requirements for those working at Biosafety Level 2
2. Read toxin-specific Best Practices document (if applicable)
3. Read all components of the toxin safety plan and complete a question & answer session with the PI or lead researcher using the toxin (i.e., toxin mentor)
4. Complete observational and hands-on training and qualification with the lab's toxin mentor for the specific procedures the person will carry out with the toxin
5. If person will be performing toxin reconstitution procedures or concentrated toxin procedures involving a sharp, a dry run must be performed with VU Biosafety or the lead researcher who is qualified to perform this procedure.

All training and qualification actions should be documented, and these records kept with the toxin safety plan. See Attachment 2 for an example of a completed training record template.

Policy Revision History

The VU IBC reviewed and revised this policy on:

January 28, 2025 to capture forthcoming regulatory changes and synchronize information related to management of this Program and other Biosafety documents. Summary of revisions includes the following:

- Updated the policy title; removed reference to venoms,
- Added of the assignment of a toxin mentor as a specific requirement for IBC registration and approval of acutely hazardous biological toxin use,
- Added passage regarding toxin use activity surveys that will be administered by VU Biosafety and expectations for PIs regarding pauses in toxin use,
- Updated information reflecting primary post-exposure treatment at the VUMC Emergency Department,
- Added informational text regarding forthcoming applicability of DURC requirements to all select agent-listed toxins.

The VU IBC reviewed proposed edits prepared by VU Biosafety and endorsed this policy on December 9, 2025. A summary of the revisions includes the following:

- Updated permissible amount for conotoxins and communication information
- Updated hyperlinks
- Updated contact information

ATTACHMENT 1 - Toxin SOP Template (4 Pages)

BIOLOGICAL TOXIN LABORATORY STANDARD OPERATING PROCEDURE	Procedure title:
Latest validation/verification completed by/date:	
Validation/verification description:	

This template is most suitable for lab procedures involving toxins such as preparation of stock solution/aliquots and administration of toxin solutions to cell cultures or in an animal model. Include sufficient detail to provide effective guidance to someone who is relatively new to your lab. **It is STRONGLY recommended that separate SOPs be developed for distinct activities.**

Procedure validation/verification should be completed:

- initially,
- before initiating significant procedural changes,
- following any mishap or near-miss, and
- before reinstating toxin work that has been suspended for more than 6 months.

Name Biological Toxin/Venom:	Vendor Name:
Product ID/Catalog Number:	Animal protocol # (if applicable):
Storage Location (Room#/Building):	Location where material will be handled:
Principal Investigator:	Department:
Phone:	Email:
Toxin Mentor:	Title:

Who will be handling this material? The following lab personnel are authorized toxin users and acknowledge that they have read this document and will adhere to all regulatory policies and safety procedures including completion of appropriate training.		
Lab Personnel Name	Personnel ID Number	Signature

Basic Description of Research Use of Materials
Provide a brief summary of the proposed use of toxin for your research purposes. Please provide quantity and format of toxin to be obtained and how this material is packaged as received. (NOTE: If dry toxin to be used is packaged in a screwtop vial instead of a septum vial, please provide a justification/rationale in your description.) Please describe the necessary manipulations including any need for the use of sharps or glass, manipulations involving dry powders, etc.

OPERATIONAL SAFETY DETAILS	REMINDERS
Where is the toxin located and how is it secured?	Toxins must be secured and accounted for at all times. They should be stored in a locked storage unit or a locked lab at all times when unattended.
Where is the inventory log and when does it need to be completed?	The log should be maintained in a location where all personnel authorized to access and use the toxin can get to it readily. Routine inventory checks should be done even if toxin is not used routinely to verify quantities on hand.
Which hood or BSC is designated for toxin use?	The hood or BSC should be posted with the "toxins in use" sign; all unnecessary items removed; working surface draped with disposable drop cloth; verify proper airflow at sash before beginning work
How is the toxin secondarily contained to prevent a spill?	Primary containers of toxin/toxin solutions should be stored in a non-breakable, rigid, leak-proof container with a secure lid for storage and movement to the hood or BSC.
What surface decontaminant can be used for treating surfaces that may have been contaminated with toxin? How is it prepared, and what is the contact time?	Assure that you have freshly prepared decon solution available in the hood or BSC to treat a spill before you start working. Also assure that you have adequate absorbent towels and forceps or tongs readily available for spill cleanup if needed.
What personal protective equipment must be worn, for what tasks, and where do you get it?	Disposable, fluid-resistant wraparound gown with elastic cuff and 2 pair of gloves configured to completely cover the wrist are recommended. Glove material should be compatible with diluents to be used.
What sharps will need to be used for this procedure?	Use a vial adapter if one is available and access to a septum vial is part of the procedure. If a needle must be used, use a syringe with a fixed or luer lock needle; use a safety engineered device that allows for enclosure of the sharp end after use for reconstitution of concentrated toxin.

OPERATIONAL SAFETY DETAILS	REMINDERS
What specific safe sharps handling techniques apply to this procedure?	All sharps handling steps should be carried out in such a way that your non-dominant hand is out of the "strike zone" of the sharp. Disposable sharps should be immediately discarded in a sharps container that is available within arm's reach.
How is solid, non-sharps waste to be collected and treated for disposal? (Waste will either be treated by autoclave or submitted to VU Chemical Waste.)	A receptacle for solid waste collection should be available inside the hood or BSC. The receptacle should have the same features as a secondary container and it should be lined with an appropriate bag. Biohazard bags should NOT be used for wastes that cannot be treated by autoclave.
Are there specific post-exposure actions to be followed as prescribed by Occupational Health? If YES, what are they?	In the event of: • a splash to the eyes, nose, mouth, or • contact with unprotected skin, or • a cut/puncture with a contaminated item Proceed to the sink and flush the affected body area for 15 minutes. Use soap and water for unprotected skin or cut/puncture exposures. Report the exposure to your supervisor if available. Proceed to the VUMC Emergency Department with a copy of their protocol (to identify the potential exposure dose). ED will consult with Occupational Health.

SUPPLIES NEEDED FOR PROCEDURE (Include or reference safety supplies mentioned in previous sections)
SET-UP & PRE-CHECKS (This should include safety practice actions such as advance notification of lab staff, hood/BSC setup, equipment checks, etc.)
PROCEDURAL STEPS

CLEAN-UP & RECORDKEEPING (Include waste collection, area decon, inventory records, etc.)

PRINCIPAL INVESTIGATOR ACKNOWLEDGEMENT OF RESPONSIBILITIES RELATED TO SELECT AGENT TOXIN POSSESSION

In order to fulfill these regulatory responsibilities, I acknowledge that:

- If there is no reason to keep the toxin, I will contact VU Biosafety in order to make arrangements for destruction of the toxin.
- If the toxin is to be maintained, it must be secured at all times. The means of security will be sufficient to prevent a person who does not work in the lab from having ready access to it.
- An inventory sheet will be maintained to document how much toxin is on hand, any use of toxin-how much, for what purpose and by whom, etc. This documentation will be maintained in such a way that is readily available in the event of regulatory inspection.
- If I plan to transfer a toxin to another PI, the VU Biosafety Officer will be contacted to prepare a transfer letter and coordinate that transfer (provided that the party is registered with and approved by the IBC). Transfers to a party outside Vanderbilt are not permitted.
- Laboratory personnel identified as authorized toxin users will be on record with Occupational Health and complete all training and qualification actions before handling toxins.

Principal Investigator Signature/Date

Please contact VU Biosafety VUBiosafety@vanderbilt.edu for assistance with questions related to biological toxin safety practices.

ATTACHMENT 2 - Training Record Template Example (2 pages)

Biological Toxin Personnel Training & Qualification Record

Toxin Procedure Mentor: Use this document to record all relevant actions and qualifying events required for the individual named to be approved for independent work with toxins of biological origin included in the lab's Toxin Safety Plan. Maintain all completed records with the Toxin Safety Plan for regulatory review purposes. Please notify the BSO whenever a person has been added to, or removed from, the roster of personnel authorized to work with the toxin.

Personnel Information			
Name:	Trainee Eligibility Requirements	Date completed	Verified by (initials)
Job Title:	Biosafety 101 (or equivalent)		
Phone & email:	Biosafety 201 (or equivalent)		
PI:	Toxin Safety Plan Read		
Assigned toxin procedure mentor:	Toxin Safety Plan Q&A with Toxin Procedure Mentor		
Toxin(s) to be worked with & scope of activities to be performed:	Addition to IBC registration		
	Medical surveillance enrollment (if applicable)		

TOXIN PROCEDURE PROFICIENCY ACKNOWLEDGMENT

_____ has satisfactorily completed all lab-specific procedural training (listed and documented on Page 2 of this form (or as outlined in attached records)). They have been observed to be proficient in carrying out all procedures as outlined in the lab's Toxin Safety Plan.

_____ _____

Name of Principal Investigator Signature & Date

_____ _____

Name of Toxin Procedure Mentor Signature & Date

AUTHORIZED TOXIN USER CODE OF CONDUCT ACKNOWLEDGMENT

I agree to follow all technical and biosafety procedures as outlined in the lab's Toxin Safety Plan. I understand that my privileges to work with _____ toxin may be revoked if I fail to follow these procedures.

_____ _____

Name of Authorized Toxin User Signature & Date

LAB-SPECIFIC HANDS-ON PROCEDURAL TRAINING AND PROFICIENCY DETERMINATION

Toxin mentor: Please complete the table below for each procedure/activity that the trainee will perform that is included in your Toxin Safety Plan once you feel they are ready to be qualified for independent work with toxin.

PROCEDURE:	Date of proficiency determination:
TRAINEE:	Determination made by:

Did trainee successfully demonstrate the following?		**YES**	**NO**	**Comments/corrective actions**
1.	Knowing the location of the toxin safety plan			
2.	Wearing appropriate attire for toxin work (long pants and footwear the covers all skin on lower extremities)			
3.	Properly wearing PPE specified in toxin safety plan for THIS procedure			
4.	Posting the area for toxin use before work begins & removing the posting once work is finished and area is decontaminated			
5.	Properly staging the area where toxin use will take place including clearing unnecessary items, checking safety equipment, staging waste collection container, and preparing disinfectant as specified in toxin safety plan for THIS procedure			
5.	Properly accessing and securing toxins in storage			
6.	Transporting toxin products in a way that prevents spills/releases and as specified in toxin safety plan for THIS procedure			
7.	Performing manipulations with toxins in a manner that minimizes the potential for dispersal of particulates or aerosols			
8.	Decontaminating all items and impacted surfaces using decontaminant and contact time specified for this toxin			
9.	Collecting all toxin wastes in proper receptacles and understanding actions for proper disposal (i.e., in-lab treatment v. submission as hazardous waste) for toxin in use			
10.	Properly logging toxin inventory and activity			
11.	Understanding actions required for responding to an exposure to the toxin in use including where to report for post exposure follow up			