BIOSAFETY BEST PRACTICES FOR RESEARCH USE OF BIOLOGICAL TOXINS & VENOMS

This document applies to the use of biological toxins and venoms that meets one or more of the following criteria:

- toxin/venom presents a life-threatening or severe irreversible health effect risk in a single exposure incident scenario;
- toxin/venom meets the OSHA definition of a particularly hazardous substance as outlined in the VEHS document “Managing Particularly Hazardous Substances”;
- toxin/venom is included on the CDC/APHIS Select Agent List.

Work with materials that present an elevated health risk requires careful planning and institutional review/approval to ensure the safety of those who are working with such materials. All attachments are available on the Toxin User’s ROCKET site (for currently approved labs), or will be provided by VEHS during initial activity review.

Institutional Review & Approval for Possession and Use of Toxins

Possession and research use of biological toxins or venoms (to be referred to simply as “toxins” for purposes of this document) requires registration with, and approval by, the Vanderbilt Institutional Biosafety Committee (IBC). In order to gain approval for this activity, the following 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 prepare a Toxin Safety Plan that includes:
   - A copy of this document;
   - Material safety data sheets for the toxin(s) to be used;
   - Mass-balance inventory for each toxin;
   - Standard operating procedures (SOPs) that incorporate relevant safety information into the procedural steps (see Attachment 1);
   - Training records for all personnel authorized to handle the toxins(s) (see Attachment 2);
   - A completed and approved Occupational Health Plan Worksheet for each toxin (see Attachment 3)
3. VEHS will meet with lab personnel who plan to use toxins in order to review the proposed procedures and provide guidance on procedural refinements and any toxin use items in need of further consideration or action. VEHS will report lab’s toxin safety plan status to the IBC and request approval on the lab’s behalf.

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). Additionally, the toxin must be secured and accounted for at all times, and the toxin cannot be transferred to another PI without notification of, and approval by, VEHS Biosafety. (NOTE: To assure that toxin quantities in possession do not surpass restricted limits, a mass- balance style inventory sheet needs to be maintained to document how much toxin was used and for what purpose.) In all circumstances, personnel who have exposure potential to biological toxins and venoms 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 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. Under routine circumstances, no one should enter the area where this work is being conducted aside from personnel authorized to work with the toxin.

All work with toxins of biological origin needs to be carried out in accordance with Appendix I of the CDC/NIH “Biosafety in Microbiological and Biomedical Laboratories”, 5th edition. Additionally, toxins and venoms meet the definition of particularly hazardous substances under the OSHA Lab Standard. In accordance with both of these standards, only personnel who have demonstrated proficiency in lab and animal handling techniques (when applicable) as well as completed chemical and biological safety training associated with the toxin use should be permitted to handle these materials. An authorized personnel training record should be maintained with the toxin safety plan. An example of the minimum training requirements is shown in the following example. A blank record is included as Attachment 2.

EXAMPLE OF TRAINING RECORD

Document the training of all personnel authorized to perform toxin procedures in the table below. The lab manager or PI should provide initials after verifying the completion of the training and that proficiency has been demonstrated.

<table>
<thead>
<tr>
<th>Name</th>
<th>Toxins authorized to use</th>
<th>Biosafety Principles</th>
<th>Toxin Safety</th>
<th>Chemical Safety</th>
<th>Proficiency demonstrated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chuck Jones</td>
<td>Tetrodotoxin</td>
<td>8/13/12 RT</td>
<td>8/15/12 RT</td>
<td>7/30/13 RT</td>
<td>10/1/12 RT</td>
</tr>
<tr>
<td>Michelle Brown</td>
<td>Diphtheria toxin</td>
<td>9/12/12 RT</td>
<td>9/30/12 RT</td>
<td>9/12/11 RT</td>
<td>10/15/12 RT</td>
</tr>
</tbody>
</table>

Exposure Hazards Associated with Toxins

Inhalation of toxin during handling of dried toxin product is a significant exposure risk. Toxin preparations need to be carried out in a biological safety cabinet (BSC) or a chemical fume hood. Animal procedures involving toxin administration should also be carried out in a BSC or fume hood whenever feasible. (Note: Remove all items that are not necessary for the procedure at hand from the BSC or hood before handling the toxin to further reduce spill/contamination potential.)

Whenever possible, avoid using a dry toxin product. If one must be used, use a premeasured product that will allow for adding of the diluent without the need to open the container and manipulate dry toxin. *(NOTE: If you MUST manipulate a dry toxin in an open vessel, you must develop specific safety procedures addressing that exposure risk in conjunction with VEHS Chemical Safety. Specific containment equipment and respiratory protection may be required.)*

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 in the event that it is dropped. To achieve this, use a rigid plastic container with a screw cap or latchable lid.
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. (A companion document addressing animal procedures is available on the VEHS website.) 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 introducing a needle through a septum, 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 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 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 VEHS 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:

- 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.

- **Solid waste items (i.e., gloves, waste vials, bench paper, etc.)** should not be soaked in a liquid decontamination solution.
  - In the event that the toxin can be inactivated by autoclave treatment, solid waste should be collected in an autoclaveable 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 VEHS document entitled: Laboratory Guide for Managing Chemical Waste. (In short, waste will be collected in a labeled, lined bucket with a lid and pink hazardous waste tag.)
- **Sharps contaminated with a toxin may be disposed of in biohazardous sharps containers.**
- **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. VEHS 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/dust pan. 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 VEHS 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 in the event that 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 Occupational Health (or the Adult Emergency Department if prescribed by the Occupational Health Plan Worksheet) for post-exposure follow-up and take any information about the source material that you have readily available along with you.

6. Notify the LAB SUPERVISOR and Biosafety Officer (BSO) at 322-0927 as soon as possible once medical follow-up actions have been initiated.
**ATTACHMENT 1 (Page 1 of 2)**

**Toxin SOP Template**

<table>
<thead>
<tr>
<th>OPERATIONAL SAFETY DETAILS</th>
<th>REMINDERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Where is the toxin located and how is it secured?</td>
<td>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.</td>
</tr>
<tr>
<td>Where is the inventory log and when does it need to be completed?</td>
<td>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.</td>
</tr>
<tr>
<td>Which hood or BSC is designated for toxin use?</td>
<td>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</td>
</tr>
<tr>
<td>How is the toxin secondarily contained to prevent a spill?</td>
<td>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.</td>
</tr>
<tr>
<td>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?</td>
<td>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.</td>
</tr>
<tr>
<td>What personal protective equipment must be worn, for what tasks, and where do you get it?</td>
<td>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.</td>
</tr>
<tr>
<td>What sharps will need to be used for this procedure?</td>
<td>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.</td>
</tr>
</tbody>
</table>
## OPERATIONAL SAFETY DETAILS

<table>
<thead>
<tr>
<th>What specific safe sharps handling techniques apply to this procedure?</th>
<th>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.</th>
</tr>
</thead>
<tbody>
<tr>
<td>How is solid, non-sharps waste to be collected and treated for disposal? (Waste will either be treated by autoclave or submitted to VEHS Chemical Waste.)</td>
<td>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.</td>
</tr>
</tbody>
</table>
| 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 Occupational Health Clinic (6th Floor Medical Arts Building) unless specific procedures indicate otherwise. |

## 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.)

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*Please contact VEHS Biosafety at 322-2057 for assistance with questions related to biological toxin safety practices. After hours, please contact 835-4965 to request assistance.*

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ATTACHMENT 2

AUTHORIZED PERSONNEL TRAINING RECORD:
ACUTELY HAZARDOUS BIOLOGICAL TOXINS

All work with toxins of biological origin needs to be carried out in accordance with Appendix I of the CDC/NIH “Biosafety in Microbiological and Biomedical Laboratories”, 5th edition. Additionally, toxins and venoms meet the definition of particularly hazardous substances under the OSHA Lab Standard. In accordance with both of these standards, only personnel who have demonstrated proficiency in lab and animal handling techniques (when applicable) as well as completed chemical and biological safety training associated with the toxin use should be permitted to handle these materials.

Document the training of all personnel authorized to perform these toxin procedures in the table below. The lab manager or PI should provide initials after verifying the completion of the training and that proficiency has been demonstrated.

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<thead>
<tr>
<th>Name</th>
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Note: All personnel should be on record with Occupational Health regarding their potential for toxin exposure. This should be done using the Occupational Health Plan Worksheet (see Attachment 3).
ATTACHMENT 3 (Page 1 of 2)
Occupational Health Plan Worksheet

Vanderbilt University

Occupational Health Plan Worksheet
for Research Involving Materials with High Acute Toxicity

This form needs to be prepared for any research use of a toxic material for which upon a single exposure incident, the exposed person would experience life-threatening or severe irreversible health effects. Exposure incidents may occur as the result of direct contact with eyes, nose, mouth, skin, accidental inhalation, or a cut/puncture with a contaminated object (including needle sticks). Examples of acutely toxic materials include: chlorine, hydrogen cyanide, MPTP, certain toxins of biological origin, certain animal venoms. The PI should complete Section 1 of this form and submit it to VEHS along with the material safety data sheet (MSDS) for the toxic material to be used and a copy of proposed safe handling procedures.

SECTION 1: TO BE COMPLETED BY PRINCIPAL INVESTIGATOR

<table>
<thead>
<tr>
<th>Name of High Acute Toxicity Material:</th>
<th>Animal protocol #:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(If applicable)</td>
</tr>
</tbody>
</table>

Reason for submission: (new protocol, adding personnel, annual renewal, other)

<table>
<thead>
<tr>
<th>Location where material will be stored:</th>
<th>Location where material will be handled:</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Principal Investigator:</th>
<th>Department:</th>
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<table>
<thead>
<tr>
<th>Phone:</th>
<th>Email:</th>
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</table>

<table>
<thead>
<tr>
<th>VEHS Safety Representative:</th>
<th>Date lab consult completed:</th>
</tr>
</thead>
<tbody>
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<td></td>
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</tr>
</tbody>
</table>

Who will be handling this material?
List all lab personnel who will handle or have direct access to this material.

<table>
<thead>
<tr>
<th>Lab Personnel Name</th>
<th>Personnel ID Number</th>
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</tbody>
</table>

Description of Research Use of Materials
Provide a brief summary of the proposed use of this material for your research purposes. Please provide quantity of material to be obtained and how this material is packaged as received. Please describe the necessary manipulations including any need for the use of sharps or glass, manipulations involving dry powders, etc.

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**ATTACHMENT 3 (Page 2 of 2)**

**Occupational Health Plan Worksheet**

<table>
<thead>
<tr>
<th>Name of high acute toxicity material:</th>
<th>PI:</th>
</tr>
</thead>
</table>

**SECTION 2: TO BE COMPLETED BY OCCUPATIONAL HEALTH**

- **Use of these research materials requires:**
  - Immunization(s) Specify: Frequency:
  - Treatment upon exposure Specify:
  - Serum collection and storage Frequency:
  - Serum analysis For what?: Frequency:
  - Medical History Frequency:
  - Medical Tests Test(s): Frequency:
  - Physical Examination Specify: Frequency
  - Respiratory Protection Type of respirator:

**Exposure to these research materials could put persons with the following health conditions at additional risk:**

**Unexplained illnesses and symptoms such as:**

*must be reported to the Occupational Health Clinic (936-0955).*

Additional documents should be appended to this document including (1) the MSDS for material to be used, and (2) a copy of safe handling procedures for lab and animal procedures.

This Occupational Health Plan must be reviewed and approved annually. The signatures below signify acceptance of this plan. A copy of the completed Plan and attached documents should be maintained by all with a signature line. Annual review/approval should be initiated by the PI at least 30 days before the approval date if they have not been otherwise contacted regarding this requirement by Occupational Health or VEHS. If the use of this toxic material is terminated and material is to be disposed of, the PI should contact the VEHS Chemical Safety Representative for assistance with disposal and close-out of this Plan.

**Signature and Date:**

_________________________ Principal Investigator

_________________________ VEHS Safety Representative

_________________________ Occupational Health Practitioner

*VEHS 6/2013*