1. Introduction

Biological toxins are toxic substances that are produced by certain species of living organisms, including microbes, plants, and animals. These substances cause disease by interfering with biological processes in affected/exposed organisms; however, they are not considered infectious or capable of causing communicable diseases because they do not replicate. Some examples of these substances include botulinum toxins, tetrodotoxin, conotoxins, immunotoxins, and venoms (e.g., snakes, scorpions, etc.).

Although these materials are produced by biological species, they may be extremely toxic in miniscule quantities. The primary risks associated with laboratory use of biological toxins in the laboratory are inadvertent exposures to these materials, which can occur through any route of exposure, including inhalation, injection through needles and other sharp objects, absorption through skin or open wounds, mucous membranes, and accidental ingestion. Depending on the toxin, concentration, quantity, and route of exposure, adverse health effects can be minor (e.g., skin/eye irritation, nausea, vomiting, etc.) to severe (e.g., respiratory distress, muscle paralysis, seizures, and ultimately, death). Therefore, when working with these materials in the laboratory, the proper training, work practices, engineering controls, and other safety measures must be implemented.

Some biological toxins are determined to have the potential to pose a severe threat to both human and animal health, to plant health, or to animal and plant products. The possession, use, and transfer of these agents are regulated by the Centers for Disease Control (CDC) and United States Department of Agriculture (USDA) through the Federal Select Agent Program. Working with biological toxins, particularly those on the select agent list, will have additional requirements through the CDC, USDA and the KSU Institutional Biosafety Committee (IBC), and Institutional Animal Care and Use Committee (IACUC).

2. Purpose

The purpose of this document is to provide general guidance for working safely with biological toxins.

3. Scope

These guidelines apply to all faculty, staff, and students who handle, store, and work with biological toxins in laboratory areas owned, leased, or operated under Kennesaw State University (KSU).

4. Responsibilities

A. Principal Investigators, Lab Supervisors, and Managers

Principal investigators (PIs), lab supervisors, and/or lab managers are responsible for the following:

- Identifying biological toxins that are being used in the lab.

- Ensuring that the proper training has been completed by all end users prior to working with biological toxins.
- Assessing the hazards associated with working with these materials, and ensuring that the proper safety measures, engineering controls, work practices, disposal methods, and emergency procedures are in place prior to working with the material.

B. Environmental Health and Safety

The Environmental Health and Safety (EHS) Department will assist the PI and lab personnel in assessing hazards associated with biological toxins, ensure that proper engineering controls are in place, and will provide guidance for establishing administrative controls, good work practice and selection of proper personal protective equipment (PPE).

5. Safety Practices and Procedures

The following safety practices and procedures must be considered for working with biological toxins.

A. Health and Safety Review

A hazard assessment must be conducted by the PI prior to any experiments and/or work activity involving biological toxins to identify all apparent safety issues and risks associated with their use.

EHS will also conduct a hazard assessment and review based on the following (not limited to):

- Proposed experimental procedures provided by the PI.
- The specific biological toxin(s) proposed for use and the associated hazards.
- A walkthrough/inspection of the laboratory where the material will be handled, used, stored, and disposed.
- Any associated regulatory requirements.

Any work involving biological toxins that cause human illness and/or disease will require review and approval by the IBC. If biological toxins will be used in experiments with animal species, IACUC review and approval will also be required.

Biological toxins that are identified as select agents/toxins are regulated by the CDC/USDA and must meet all requirements of the CDC/USDA Federal Select Agents Program.

B. Planning

Careful planning needs to precede any work involving biological toxins. This includes initial work with the material, or whenever a change in protocol/procedures could possibly add safety issues or increase the risk of exposure.

Planning should include consultations with EHS, or colleagues who have experience in handling the substance safely and in protocols being used.

- Submit an SDS and/or other information about the toxin (if available).

- Determine if the biological toxin proposed for use is identified as a Select Agent/Toxin. Consult with EHS to determine all regulatory requirements, and if the appropriate biosafety levels and controls are available to support the work.
- Submit work involving biological toxins that cause human illness or disease to the IBC and IACUC (if involving animal species) for review and approval.

Effective planning should always consider the substitution of biological toxins with less hazardous alternatives that will achieve the desired result.

When working with biological toxins, the smallest amount of material that is practicable for the purpose of the experiment/process should be used.

Prior to beginning work with a biological toxin, coordinate with local medical facilities in the area to ensure that the appropriate antidote/antivenom available and accessible in the event of an exposure. If the antidote/antivenom is not readily available, it is important to determine from where it can be obtained, and in what time frame.

If there is no available antidote/antivenom for the toxin, or if it cannot be obtained within 2-4 hours of the exposure, working with the toxin is not recommended.

C. Training

All lab personnel who will work with biological toxins must complete all required safety training.

All lab personnel must read the safety data sheet (SDS) prior to working with the material. The SDS must always be available to current and future end users for reference.

The PI must provide laboratory specific training for working with the material.

Practice drills or "practice runs" of the experimental procedures using non-hazardous materials are highly recommended before working with the actual toxin.

Ensure that all completed training is documented.

D. Laboratory Procedures

The CDCs Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition, Appendix I states that each laboratory that works with toxins must develop a toxin-specific chemical hygiene plan.

Standard Operating Procedures (SOP) that describe (in detail) the experiment/process and identify the hazards and safeguards that will be used in all phases of the experiment/process must be in place before the work begins. The SOP must include (not limited to) the following:

- Health and safety implications associated with the handling, storage, and use of the material.
- Proper use of sharps, pipettes, or other means of transferring material
- The appropriate biosafety level for working with the material and the associated practices with the biosafety level.
- Proper engineering controls required for working with the material.
- Proper PPE that must be worn.

- Appropriate signage and labeling required.
- Proper waste production, collection, and disposal.
- Decontamination/deactivation of contaminated materials.
- Emergency spill procedures.
- Emergency procedures for exposures, accidents, and injuries.

EHS can assist with the review of the SOP to ensure that all appropriate components have been included. If needed, EHS can provide an SOP Template to use as a guide. Ensure the SOP is available to all current and future end-users for reference.

E. Designated Areas

Activities involving biological toxins, including their transfer to and from containers, must be confined to a designated work area in the laboratory.

A functional chemical fume hood, glove box, or biosafety cabinet must be used. **A functional, fully enclosed glove box is recommended.** The use of laminar flow hoods is not appropriate for working with these materials, as they do not protect against worker exposure.

Conspicuous signs, as shown below, should be posted in the designated work area.

DANGER!
DESIGNATED AREA
BIOLOGICAL TOXIN(S) IN USE
AUTHORIZED PERSONNEL ONLY

When toxins are being used inside a lab space, the door should be posted with a sign that clearly states "TOXIN(S) IN USE – AUTHORIZED PERSONNEL ONLY," or similar verbiage.

All employees working in the lab must be educated on the hazards associated with biological toxin use and controls required for the materials being used.

EHS, in consultation with lab supervisor/PI, will determine which procedures need to be confined to designated areas.

F. Access Control

The lab or work area where biological toxins are used and stored must be secure, and away from public access.

Key-Card access to the lab or work area where these materials are stored should be limited to certain individuals (e.g., lab personnel, KSU Police, EHS, etc.).

Stock solutions and working solutions of the materials should be properly labeled and stored secondary containment that can be closed and/or sealed.

It is strongly recommended that the material be stored in a locked box or cabinet with access limited to only personnel trained to work with the material.

Biological toxins should not be used by or transferred to any other researcher or personnel of any other laboratory.

G. Safe Work Practices

Working with biological toxins requires more tightly controlled work practices and operating procedures in the laboratory. The following work practices must be considered.

- Experiments with biological toxins should be conducted at **Biosafety Level 2 (BSL2)** containment and practices, at minimum.

  While some biological toxins may require BSL3 or higher containment, KSU does not currently support biosafety containment above BSL2. Before working with any biological toxin or infectious agent, contact EHS to confirm the appropriate containment level is available and implemented.

  - Each experimental procedure should be conducted using the minimum amount of the substance, consistent with the requirements of the work.
  - A working inventory of biological toxins should be kept in a logbook and stored with the material. The volume/quantity of each toxin should be documented upon receipt with the date received and the signature of the individual who accepted it. Each entry in the logbook should indicate the amount of toxin used, the purpose for use, and should be dated and signed by the end user.
  - Stock materials should be stored in leak proof, shatter resistant primary containment, and leak proof, shatter resistant secondary containment.
  - Label containers with the toxin name, concentration, hazard (toxic), and the date prepared.
  - Label all waste containers as, "Hazardous Waste." Include the toxin name and any other contents and the hazard(s).
  - Any transport of biological toxins must be in properly labeled, leak/spill-proof, non-breakable secondary containers. These materials must not be transported in personal vehicles, on university shuttles, or other public transportation.
  - Biological toxins must never be smelled or tasted.
  - A functional chemical fume hood, glove box, or biosafety cabinet must be used when working with biological toxins. **A functional, fully enclosed glove box is recommended.** Prior to beginning work, the end-user should verify that the containment device being used is functioning properly.
  - If opening a tube or vial of the material, only do so inside a functional chemical fume hood, glove box, or biosafety cabinet.
- The use of laminar flow hoods is not appropriate for working with these materials, as they do not protect against worker exposure.

- Employ safe sharps procedures when manipulating the material.
- The use of locking or integrated needle syringes or disposable syringe units are strongly recommended.
- Use vial adapters when feasible 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.
- Ensure that an appropriate sharps container is available in the immediate vicinity of the experiment for proper disposal.
- Do not recap syringes, but rather dispose of them directly into the available sharps container after use.
- When possible, use a needle-safe devices (i.e., the sharp end is retractable or enclosed after use).
- Wear the appropriate PPE when working with biological toxins.
- Working with dry biological toxin material (e.g., powder, freeze dried, etc.) can significantly increase potential exposure to the toxin through inhalation and surface contamination. For these reasons, the use of dry material is discouraged. However, if working with dry material cannot be avoided, it is strongly recommended that a pre-measured amounts be ordered that will allow dilution without opening the container.

If work with dry forms of biological toxins must be done in open vessels, specific written, safety measures must be created that will explain how to minimize inhalation exposure and contamination of work surfaces. Personnel must then be trained on these procedures. Respiratory protection may also be required. In this case, contact EHS for guidance on developing safety procedures.

- Work with biological toxins in only the conspicuously marked, designated area.
- If using toxins during animal studies, restrain or sedate animals as needed.
- Wipe any drips/residues from containers and work surfaces with absorbent material (e.g., paper towel, Kimwipes™, etc.) and dispose of the in the appropriate hazardous waste stream. It is recommended to layer work surfaces with absorbent bench top paper with a moisture-proof lining, or other impervious material to facilitate decontamination.
- Containers of toxins should be removed from the containment device (i.e., chemical fume hood, BSC, or glove box) only after the exterior of the primary container has been decontaminated.

Once the primary container is decontaminated, it should be placed in a clean secondary container.

- Upon completion of the operation, decontaminate or discard the protective covering material as hazardous waste.
- Disposable PPE such as gloves or disposable aprons should be removed in the designated area and discarded in the appropriate hazardous waste stream.
- Equipment (including reusable personal protective equipment such as face shields or goggles) that might be contaminated must never be removed from the designated area without complete decontamination.
- Wash hands thoroughly with soap and warm water before leaving the work area and prior to consuming food/beverages.
- Individuals working with biological toxins must never work in the lab alone. At least one other person should be present in case of accidents or emergencies.
- Eating, drinking, smoking, chewing gum, instilling contact lenses, applying cosmetics, and storage of food, beverages, and tobacco products is prohibited in laboratory work areas where biological toxins are handled, stored, and used.
- Practical jokes or other behavior that might confuse, startle, or distract another worker are prohibited.

H. Personal Protective Equipment

Working with biological toxins require the use of PPE. Before working with the material, always read the SDS (if available) or consult EHS for guidance on the appropriate PPE.

- At a minimum, tight fitting chemical splash goggles, laboratory coats (or disposable gowns), appropriate gloves, and closed toed shoes should be worn when handling biological toxins.
- In some instances, face shields in addition to safety goggles may be required. Eye protection must be worn even when working inside of a chemical fume hood, biosafety cabinet, or glove box.
- Gloves must be selected based on the material being used. Fluid resistant, disposable gloves (i.e., nitrile, etc.) should be worn for manipulations with biological toxins. Double gloving is strongly recommended as long as it does not affect the safe handling and use of the material.
- Some applications may require the use of respiratory protection. The use of respiratory protection requires a hazard assessment, training and medical evaluation, and respirator fit testing through EHS.

6. Decontamination/Inactivation of Toxins and Waste Disposal

The CDC's BMBL, 6th edition defines decontamination of a biological toxin as being rendered inactive and no longer capable of exerting its toxic effect. As all biological toxins are chemically and structurally unique, there is no universal method for inactivation of all biological toxins.

According to the BMBL, most toxins are susceptible to steam inactivation (i.e., autoclaving) or chemical inactivation (e.g., sodium hydroxide solution, sodium hypochlorite solution, etc.). However, the inactivation method must be specific for each toxin, and no inactivation method should be assumed to be 100% effective unless validated through sampling and testing. Appendix I (Tables 1 and 2) in the BMBL provides more specific information for the inactivation of select toxins (those on the select agent list), but it is recommended that inactivation measures for other toxins be confirmed through published research or testing. It is important for researchers who use toxins to include this information in their standard operating procedures and to cite the source of literature. If no definite means of decontamination can be identified, the material must be properly packaged and disposed of as biohazardous waste for incineration.

A. Waste Disposal

Inactivate stock vials of toxin or waste toxin using the appropriate chemical or physical method for the specific toxin (i.e., as identified in studies or other literature).

Toxins in stock vials or waste toxin inactivated by bleach may be disposed of down the lab sink. Toxins inactivated through other chemical means should be disposed of through EHS as chemical waste.

When using bleach solutions to deactivate toxins (and other biohazards), they are only effective for 24 hours. A new bleach solution must be mixed each day.

All solid waste materials, including gloves, disposable aprons or gowns, bench paper, waste vials, etc. should be treated using the appropriate physical inactivation method (e.g., autoclaving, dry heat, etc.). These materials should not be immersed or soaked in liquid chemicals for treatment.

If no definite inactivation method has been identified for the toxin, the waste should first be chemically or physically treated using conventional methods (e.g., autoclaving, chemical treatment, etc.)

- Treated stock vials or waste toxin should be absorbed onto a solid material (e.g., absorbent pad, paper towel, etc.), which can be then disposed of along with the other solid materials from the experiment.
- These materials should be properly packaged as biohazardous waste for pickup by EHS.

Contact EHS for instructions on how to properly package this waste material.

Biological toxins that are mixed with chemicals must be collected in appropriate waste containers and must be kept closed unless adding waste. The containers must be labeled, “Hazardous Waste,” and must include the contents and associated hazards. This waste must be disposed of through EHS.

Sharps used in the experiment(s) should be disposed of directly into the available sharps container. Once ¾ of the way full, the sharps container should be sealed and packaged as biohazard waste for pickup by EHS.

7. Spill Procedures

If a spill occurs during the use of a biological toxin, immediate action is necessary. Only individuals who are trained, experienced in working with the material, and are comfortable with cleaning up spills should attempt to do so.

If the spilled toxin is in solution, use the following steps:

- Isolate the area and inform everyone present that a spill has occurred. Since toxins must be used in a designated area such as a chemical fume hood or BSC, and only by trained personnel, isolation should be accomplished easily.
- Don the appropriate PPE (i.e., splash goggles, double gloves, lab coat or disposable gown) if it is not already being worn.
- Remove any broken glass (e.g., from a broken glass vial) with tongs, and dispose of in the available sharps container.
- Remove the breached container and any other non-glass items contaminated with the toxin and place in a red (biohazard) bag lined secondary container for treatment and disposal.
- Cover the spill with absorbent material (e.g., absorbent pads, paper towels, etc.) saturated with the proper inactivation agent for the toxin and allow the proper contact time with the material. If no specific inactivation agent is available, use absorbent material saturated with bleach and allow contact time of at least ten minutes.
- Absorb the liquid (use more dry absorbent materials if necessary) and remove the contaminated materials from the surface. Place the materials in the red bag lined secondary container. Use tongs or other removal devices to limit direct contact with the contamination, if feasible.

  Avoid touching surfaces during this process, as the disposable gloves are likely to be contaminated. It may be necessary to change gloves during the process.

- Apply the proper inactivation agent to the affected surfaces, including the areas near the spill that may have been affected by splashing. Allow the proper contact time for inactivation.
- Wipe away all residues with absorbent materials and allow to air dry.
- Remove all disposable PPE (e.g., gloves, gowns, etc.) and discard into the red bag lined secondary container with the other contaminated materials.
- Don more disposable gloves.
- Gather and tie the red bag in a single knot. Apply the inactivation agent to the outside of the bag dispose of in a biohazard box for disposal by EHS.
- Report the incident to EHS and complete an incident report through the Reliance system.

A. Spills of Dry Toxin Material

If dry toxin material (i.e., in powdered or freeze-dried form) is spilled inside of a BSC, glove box or chemical fume hood, it is appropriate to use the same steps used for cleaning liquid toxin spills. However, if the spill occurs outside of a chemical fume hood, BSC, or glove box the following steps must be taken.

- Do not attempt to clean up the spill. Evacuate and instruct other personnel to evacuate the lab immediately to avoid possible inhalation exposure.

- Dial extension 6666 or 470-578-666 (KSU police) to dispatch emergency responders.
- Provide emergency responders with as much information as possible, including (not limited to):
    - The biological toxin that was spilled, the concentration, physical form, and amount spilled.
    - The safety data sheet for the material, if available.
    - The names of the individuals who were present when the spill occurred.
    - The emergency contact information of the PI, researcher, or lab manager, if not present at the time.
    - The location of the spill.
- Watch for symptoms associated with exposure to the toxin; if symptoms occur, seek medical attention immediately.
- Report the incident to EHS as soon as possible and complete an incident report through the Reliance system.

8. Emergency Procedures

- In the event of any exposure to biological toxins, dial extension 6666 or 470-578-6666 (KSU Police Department) immediately to dispatch emergency responders.
- Provide the emergency responders with detailed information about the exposure:
    - Specific biological toxin and the species of origin.
    - Concentration of the toxin.
    - The quantity of material.
    - Time of exposure.
    - Number of individuals exposed.

This information will help the emergency responders determine the availability of the appropriate treatment (i.e., the antidotes/antivenoms), if the treatment is available, and the nearest location where treatment is accessible.

- If there is an exposure to the eyes, flush eyes at the emergency eyewash station immediately for 15 minutes.
- If there is an exposure to the skin, wash the affected area with soap and warm water for 15 minutes.
- If there is an inhalation exposure, immediately move the individual to fresh air, then seek immediate medical attention.
- If accidentally swallowed, immediately contact the poison control center, and seek immediate medical attention.
- If accidentally injected, seek immediate medical attention. Remain calm (or instruct the exposed individual to remain calm, and avoid excess movement). Do not apply a tourniquet, cut the site of injection, or immerse in water, as these actions may prove more harmful.
- In the event of a fire, evacuate the building immediately and dial extension 6666 or 470-578-6666 (KSU Police Department) to report the emergency. Also, notify EHS as soon as possible at 470-578-3321.