Biological Toxin Safe Work Practices

I. INTRODUCTION

Biological toxins are poisonous substances produced by certain microorganisms, animals, and plants. Examples of toxins of biological origin include Diphtheria Toxin, Tetrodotoxin, Pertussis Toxin, Botulinium Toxin, Snake Venom Toxins, Conotoxin and Ricin. Although toxins are derived from biological materials, they do not replicate and are therefore not considered infectious. However, they may be extremely toxic in very small quantities and must be managed like hazardous chemicals in the workplace. Controls must be in place to ensure staff is protected from exposure. The routes of exposure include inhalation, eye, nose and mucous membrane contact, percutaneous, and skin absorption depending on the diluents used. The main issues of concern in the laboratory are accidental exposures to toxin caused by contact with the mouth, eye, skin and mucous membranes, inhalation of toxin powder or aerosol inadvertently generated, or by needlestick incidents.

Work with toxins of biological origin must be included in your laboratory-specific Chemical Hygiene Plan. Documented toxin-specific hazard training and training on the laboratory-specific standard operating procedures (SOP) is required for all laboratory personnel prior to starting work. The training must include but is not limited to the health and physical hazards of the toxin, signs and symptoms associated with exposure, appropriate work practices, personal protective equipment, and emergency procedures.

Some toxins of biological origin are considered Select Toxins, which the US Departments of Health and Human Services (HHS) and Agriculture (USDA) have determined to have the potential to pose a severe threat to public health and safety, to animal or plant health, or to animal or plant products. The Centers for Disease Control and Prevention (CDC) in their Select Agent Program regulates the possession, use, and transfer of these specific biological agents and toxins. Research work with CDC listed Select Toxins may have additional safety and security requirements including registration with UW EH&S and the CDC.

II. LABORATORY PLANNING AND PREPARATION FOR USE

1. If working with a Select Toxin, you are required to register your work with the EH&S Research and Occupational Safety, Biosafety Programs, 206-221-7770. Certain toxin forms and toxins used in minimal quantities may be excluded from the requirements of Select Agent Regulations. See the following CDC link for the current list of Select Toxins, exclusions and exempt quantities. http://www.selectagents.gov
2. Develop a written laboratory-specific SOP specific to the toxin being used. A template Biological Toxin SOP with training documentation form is available. A diphtheria toxin SOP template is also available.
3. Provide and document hazardous chemical training and specific toxin SOP training to personnel working with toxins and any other personnel authorized or required to be

in the laboratory during toxin work. A sample training documentation form is included in the template SOP referenced above.

4. Ensure the toxin Material Safety Data Sheet/Safety Data Sheet (MSDS/SDS) is available to staff at all times and that the toxin inventory for the laboratory is entered into the UW MyChem system.
5. Designate toxin storage area in a locked container (freezer, refrigerator, cabinet or other container) in a secure location.
6. Designate a laboratory, work space, and certified biological safety cabinet (BSC), fume hood, glove box or other approved containment for toxin work. The laboratory facilities required may vary based on the level of hazard posed by the specific toxin and the procedures being performed. Work with Select Toxins may require rooms with controlled access.
7. Prepare a door sign stating "Toxins in Use - Authorized Personnel Only."
8. Post the EH&S Exposure Response Poster in the laboratory.
9. If possible, do not work with toxin in solid or powder form. If it is necessary to purchase it in powder or solid form, purchase pre-diluted or pre-weighed toxin in the minimum quantity needed to perform work. Additional precautions may be needed if working with powder or solid toxin.
10. Determine the appropriate chemical and/or physical inactivation method(s) for the specific toxin (refer to toxin inactivation Section VIII). Ensure supplies for inactivation of toxin are available.
11. Ensure supplies for spill cleanup are appropriate for the specific toxin, maintained in a clearly marked spill cleanup kit and readily available in the laboratory.

III. ENGINEERING CONTROLS

1. Designate a certified BSC, fume hood, glove box or other approved containment. Do not use a laminar flow hood or cabinet for toxin work. Consider the properties of the specific toxin and procedures when selecting a containment device.
2. In-line HEPA filters are required if vacuum lines are used with toxin.
3. If centrifuging materials containing toxin, centrifuge safety cups or sealed rotors must be used and the outside surfaces routinely decontaminated. Open the sealed cups or rotors inside containment.

IV. PERSONAL PROTECTIVE EQUIPMENT (PPE)

1. Wear safety glasses with side shields or goggles.
2. Wear a laboratory coat with long sleeves, smock, apron, or coveralls. Consider using disposable PPE.
3. Wear gloves that are impervious to the toxin as well as the diluent. Double gloving is recommended. Change gloves immediately if contaminated, torn, or punctured and dispose immediately after removal.
4. Wear face protection, such as a face shield, when splash/splatter is possible.
5. Respiratory protection (requires enrollment in UW's respirator program) may be required if an airborne hazard is present when work is done outside of approved

containment. Contact EH&S at 206-616-3777 for information or see the EH&S Respiratory Protection webpage.

V. TOXIN USE PRACTICES (reconstitution, dilution, administration)

1. Post sign on room door when toxins are in use stating "Toxins in Use - Authorized Personnel Only."
2. Work with toxins in designated rooms at pre-determined bench areas.
3. Biosafety Level 2 (BSL-2) practices are appropriate for most toxin work. However, some toxins or procedures may require additional BSL-3 practices.
4. Work with toxin in a BSC, fume hood, glove box or other approved containment.
5. Transport toxins only in labeled, leak/spill-proof, non-breakable secondary containers.
6. Perform preparations over plastic backed absorbent pads. Dispose of pads after completion of tasks or immediately upon contamination.
7. Utilize safe sharps procedures (i.e. sharps container in the immediate vicinity). Needle locking syringes or disposable syringe needle units are recommended and should be disposed of promptly after use.
8. Restrain or anesthetize animals when possible.
9. Decontaminate containers before they are removed from the fume hood, BSC, or glove box. Also decontaminate the exterior of the closed primary container and place it in a clean secondary container.
10. Decontaminate the BSC or approved containment and all surfaces used upon completion of tasks with appropriate inactivating agent and contact time.
11. All potentially contaminated disposable items (such as gloves used in preparation) must be placed in a hazardous waste bag and autoclaved before disposal.
12. Wash hands upon completion of tasks.

VI. TOXIN SPILL CLEANUP

Toxin spills must be cleaned up immediately by properly protected and trained personnel.

Liquid Spills

1. The required PPE for cleaning up spills includes a lab coat or smock, goggles, and two pairs of nitrile gloves.
2. For chemical inactivation, cover spill with absorbent paper towels and inactivate by applying the appropriate chemical inactivating agent starting at the perimeter and working toward the center, allowing prescribed contact time before clean up. Clean the spill area with inactivating agent, allowing prescribed contact time, then soap and water. The inactivated spill waste can be double bagged and disposed of in regular trash. See toxin inactivation section VIII below.
3. For physical inactivation use absorbent paper towels to wipe up liquid. Place waste in hazardous waste plastic bag and autoclave. Clean the spill area with inactivating agent, allowing prescribed contact time, then soap and water. The inactivated spill

waste can be double bagged and disposed of in regular trash. See toxin inactivation section VIII below.

Powder Spills inside a BSC or containment

1. The required PPE for cleaning up spills includes a lab coat or smock, goggles, and two pairs of nitrile gloves.
2. Gently cover powder spill with dampened absorbent paper towels to avoid raising dust.
3. For chemical inactivation, apply the appropriate chemical inactivating agent starting at the perimeter and working toward the center, allowing prescribed contact time before clean up. Clean the spill area with inactivating agent, allowing prescribed contact time, then soap and water. The inactivated spill waste can be double bagged and disposed of in regular trash. See toxin inactivation section VIII below.
4. For physical inactivation, use dampened paper towels to wipe up spill. Place waste in hazardous waste plastic bag and autoclave. Clean the spill area with inactivating agent, allowing prescribed contact time, then soap and water. The inactivated spill waste can be double bagged and disposed of in regular trash. See toxin inactivation section VIII below.

Powder spills outside of a BSC, fume hood, glove box or approved containment

1. Remove all personnel from the laboratory and restrict access; do not attempt to clean up the spill.
2. As soon as possible report the spill by notifying EH&S (EH&S business hours 206-543-0467, outside business hours 911); tell them that a spill has occurred, and you need EH&S to obtain a spill cleanup contractor.
3. Be prepared to provide the following information:
   - Name and phone number of knowledgeable person that can be contacted
   - Name of toxin, concentration and amount spilled, liquid or solid type spill
   - Number of injured, if any (refer to Section VII Acute Exposure)
   - Location of spill

   This information can also be used in reporting to the Emergency Department (ED) after potential exposure. The involved person or supervisor is required to complete and submit the Online Accident Reporting System (OARS) form to EH&S within 24 hours of any spill incident at http://www.ehs.washington.edu/ohsoars/index.shtm. For questions on spill cleanup, contact EH&S spill consultants at 206-543-0467 for guidance.

VII. ACUTE EXPOSURE

Follow the steps below for any exposures to biotoxins. These steps are also given on the EH&S Exposure Response Poster that is posted in the laboratory.

1. PERFORM FIRST AID	
Needlestick, sharps injury, puncture wound, or animal bite/scratch	Wash exposed area thoroughly for 15 minutes with warm water and sudsing soap.
Eye exposure	Use eye wash to flush eyes for 15 minutes while holding eyes open.
Skin exposure	Use nearest safety shower for 15 minutes. Stay under shower and remove clothing. Use a clean lab coat or spare clothing to cover.
Inhalation	Move out of contaminated area and get help.
2. GET MEDICAL HELP	
For chemical or radiological exposure or emergency:	Call 911 and follow the instructions given. Bring to the ED the SDS and SOP for specific biotoxin. Additional medical advice can be obtained from the Washington Poison Center at 1-800-222-1222.
For biological and all other exposures:	During business hours (Monday thru Friday 8 a.m. to 5 p.m.): • Call the Employee Health Center at 206.685.1026. • Harborview sites call 206.744.3081. If the Employee Health Center is closed: • Call 911 and follow the instructions given. Notify your supervisor. Secure the area before leaving.
3. REPORT THE INCIDENT	
In the event of hospitalization or fatality, notify EH&S immediately after first aid and getting help:	During business hours (Monday thru Friday 8 a.m. to 5 p.m.): • Call the EH&S main phone line at 206.543.7262. Outside of business hours: • Call 206.685.UWPD (8973) to reach EH&S staff on call.
All incidents and near misses:	Submit a report via the UW Online Accident Report (OARS) within 24 hours at https://oars.ehs.washington.edu

Note: For Diphtheria Toxin exposures, refer to the EH&S SOP template for Diphtheria Toxin in the EH&S Laboratory Safety Manual for specifically required Emergency Department procedures. http://www.ehs.washington.edu/resource/diphtheria-toxin-sop-547

VIII. INACTIVATION AND DISPOSAL

According to the CDC, inactivation of a biotoxin means to render the toxin non-functional so that it is no longer capable of exerting its toxic effect. This is different from inactivation of biological agents, which renders the agent non-viable, or no longer capable of growing, replicating, infecting, or causing disease. Inactivation methods used for biotoxins must be specific for the toxin, published and validated, or developed and validated with thorough testing. Note that disinfecting solutions and products may not inactivate biotoxins.

1. Inactivate any waste toxin chemically or physically (usually autoclaving) before disposal or given to EH&S for disposal.
2. Place any used PPE and spill cleanup debris in a hazardous waste plastic bag and autoclave.
3. For mixed waste (i.e. toxin waste mixed with radioactive waste) consult EH&S Radiation Safety at 206-543-0463 for disposal instructions.
4. If in-lab inactivation is not possible for some toxin waste, manage waste as hazardous chemical waste. Be aware that some form of treatment in the lab may be required before collection as chemical waste. Contact EH&S Environmental Programs Office at 206-616-5835 for disposal instructions. See Hazardous Chemical Waste Disposal for information on how to request collection of hazardous chemical waste. https://www.ehs.washington.edu/chemical/hazardous-chemical-waste-disposal
5. Dispose of liquid inactivated biotoxin waste within a pH range of 5.5 to 12 down the regular sewer drain.
6. Refer to the information below in Tables 1 and 2 on inactivation of selected toxins, which is taken directly from the publication Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition. Centers for Disease Control and Prevention. Appendix I: Guidelines for Work with Toxins of Biological Origin.
7. If using bleach solutions, prepare fresh daily for inactivation of biotoxins and decontamination of surfaces. Undiluted, commercially available bleach solutions typically contain 3 – 6% (w/v) NaOCl (sodium hypochlorite).
8. Since **Diphtheria Toxin** is not included in the BMBL tables, a review was made of inactivation methods for Diphtheria Toxin at various research institutions. The most common physical inactivation method was steam autoclaving at 121°C for 60 minutes. Although no consensus was apparent for a specific chemical inactivation agent and concentration, the commonly used chemicals included 1% NaOCl, 10% bleach, 1N NaOH, and combinations of NaOCl and NaOH. A 30-minute contact time was allowed to complete inactivation.

TABLE 1: PHYSICAL INACTIVATION OF SELECTED TOXINS

TOXIN	STEAM AUTOCLAVE	DRY HEAT (10 MIN)	FREEZETHAW	GAMMA IRRADIATION
Botulinum neurotoxin	Yes[a]	> 100ºC[b]	No[c]	Incomplete[d]
Staphylococcal Enterotoxin	Yes[e]	> 100ºC; refolds[f]	No[g]	Incomplete[h]
Ricin	Yes[i]	> 100ºC[i]	No[j]	Incomplete[k]
Microcystin	No[l]	> 260ºC[m]	No[n]	ND
Saxitoxin	No[l]	> 260ºC [m]	No[n]	ND
Palytoxin	No[l]	> 260ºC[m]	No[n]	ND
Tetrodotoxin	No[l]	> 260ºC[m]	No[n]	ND
T-2 mycotoxin	No[l]	> 815ºC[m]	No[n]	ND
Brevetoxin (PbTx-2)	No[l]	> 815ºC[m]	No[n]	ND

Table 1 Notes: ND indicates "not determined" from available decontamination literature.

[a] Steam autoclaving should be at ≥121°C for 1 h. For volumes larger than 1 liter, especially those containing *Clostridium botulinum* spores, autoclave at ≥121°C for 2 h to ensure that sufficient heat has penetrated to kill all spores.

[b] Exposure to 100°C for 10 min. inactivates BoNT. Heat denaturation of BoNT as a function of time is biphasic with most of the activity destroyed relatively rapidly, but with some residual toxin (e.g., 1-5%) inactivated much more slowly.

[c] Measured using BoNT serotype A at -20°C in food matrices at pH 4.1-6.2 over a period of 180 days.

[d] Measured using BoNT serotypes A and B with gamma irradiation from a Co source.

[e] Protracted steam autoclaving, similar to that described for BoNT, followed by incineration is recommended for disposal of SE-contaminated materials.

[f] Inactivation may not be complete depending upon the extent of toxin re-folding after denaturation. Biological activity of SE can be retained despite heat and pressure treatment routinely used in canned food product processing.

[g] SE toxins are resistant to degradation from freezing, chilling or storage at ambient temperature. Active SEB in the freeze-dried state can be stored for years.

[h.] References [15,16] in BMBL

[i] Dry heat of ≥100ºC for 60 min in an ashing oven or steam autoclave treatment at >121ºC for 1 h reduced the activity of pure ricin by >99%. Heat inactivation of impure toxin preparations (e.g. crude ricin plant extracts) may vary. Heat-denatured ricin can undergo limited refolding (<1%) to yield active toxin. Ricin holotoxin is not inactivated significantly by freezing, chilling or storage at ambient temperature. In the liquid state with a preservative (sodium azide), ricin can be stored at 4ºC for years with little loss in potency.

[k] Irradiation causes a dose-dependent loss of activity for aqueous solutions of ricin, but complete inactivation is difficult to achieve; 75 MRad reduced activity 90%, but complete inactivation was not achieved even at 100 MRad. Gamma irradiation from a laboratory Co source can be used to partially inactivate aqueous solutions of ricin, but dried ricin powders are significantly resistant to inactivation by this method.

[l] Autoclaving with 17 lb pressure (121-132 ^{o}C) for 30 min failed to inactivate low molecular weight (LMW) toxins. All burnable waste from LMW toxins should be incinerated at temperatures in excess of 815°C (1,500 ^{o}F).

[m] Toxin solutions were dried at 150 ^{o}C in a crucible, placed in an ashing oven at various temperatures for either 10 or 30 min, reconstituted and tested for concentration and/or activity; tabulated values are temperatures exceeding those required to achieve 99% toxin inactivation.

[n] LMW toxins are generally very resistant to temperature fluctuations and can be stored in the freeze-dried state for years and retain toxicity.

TABLE 2: CHEMICAL INACTIVATION OF SELECTED TOXINS

TOXIN	NAOCL (30 MIN)	NAOH (30 MIN)	NAOCL + NAOH (30 MIN)	OZONE TREATMENT
Botulinum neurotoxin	> 0.1%[a]	>0.25 N	ND	Yes[b]
Staphylococcal enterotoxin	> 0.5%[c]	>0.25 N	ND	ND
Ricin	> 1.0%[d]	ND	>0.1% + 0.25N [e]	ND
Saxitoxin	≥ 0.1%[e]	ND	0.25% + 0.25N [e]	ND
Palytoxin	≥ 0.1%[e]	ND	0.25% + 0.25N [e]	ND
Microcystin	≥ 0.5%[e]	ND	0.25% + 0.25N [e]	ND
Tetrodotoxin	≥ 0.5%[e]	ND	0.25% + 0.25N [e]	ND
T-2 mycotoxin	≥ 2.5%[e,f]	ND	0.25% + 0.25N [e]	ND
Brevetoxin (PbTx-2)	≥ 2.5%[e,f]	ND	0.25% + 0.25N [e]	ND

Table 2 Notes: ND indicates "not determined" from available decontamination literature.

[a] Solutions of NaOCl (≥0.1%) or NaOH (>0.25 N) for 30 min inactivate BoNT and are recommended for decontaminating work surfaces and spills of *C. botulinum* or BoNT. Chlorine at a concentration of 0.3-0.5 mg/L as a solution of hypochlorite rapidly inactivates BoNT (serotypes B or E tested) in water. Chlorine dioxide inactivates BoNT, but chloramine is less effective.

[b] Ozone (>2 mg/L) or powdered activated charcoal treatment also completely inactivate BoNT (serotypes A, B tested) in water under defined condition.

[c] SEB is inactivated with 0.5% hypochlorite for 10-15 mi.

[d] Ricin is inactivated by a 30 min exposure to concentrations of NaOCl ranging from 0.12.5%, or by a mixture of 0.25% NaOCl plus 0.25 N NaOH.[17] In general, solutions of 1.0% NaOCl are effective for decontamination of ricin from laboratory surfaces, equipment, animal cages, or small spills.

[e] The minimal effective concentration of NaOCl was dependent on toxin and contact time; all LMW toxins tested were inactivated at least 99% by treatment with 2.5% NaOCl, or with a combination of 0.25% NaOCl and 0.25N NaOH.

[f] For T-2 mycotoxin and brevetoxin, liquid samples, accidental spills, and non-burnable waste should be soaked in 2.5% NaOCl with 0.25% N NaOH for 4 h. Cages and bedding from animals exposed to T-2 mycotoxin or brevetoxin should be treated with 0.25% NaOCl and 0.025 N NaOH for 4 h. Exposure for 30 min to 1.0% NaOCl is an effective procedure for the laboratory (working solutions, equipment, animal cages, working area and spills) for the inactivation of saxitoxin or tetrodotoxin. Decontamination of equipment and waste contaminated with select brevetoxins has been reviewed.

Alternate methods of chemical decontamination: 1 N sulfuric or hydrochloric acid did not inactivate T-2 mycotoxin and only partially inactivated microcystin-LR, saxitoxin, and brevetoxin (PbTx-2). Tetrodotoxin and palytoxin were inactivated by hydrochloric acid, but only at relatively high molar concentrations. T2 was not inactivated by exposure to 18% formaldehyde plus methanol (16 h), 90% freon-113 + 10% acetic acid, calcium hypochlorite, sodium bisulfate, or mild oxidizing.[17] Hydrogen peroxide was ineffective in inactivating T-2 mycotoxin. This agent did cause some inactivation of saxitoxin and tetrodotoxin, but required a 16 h contact time in the presence of ultraviolet light.

IX. TRANSFER OF SELECT TOXINS

Documentation is required for any transfer of the following Select Toxins shown below in any amount (intramurally or extramurally), to any entity or individual. If you plan to transfer any of these Select Toxins you must complete a UW Select Toxin Transfer Due Diligence Form and submit a copy to EH&S Research and Occupational Safety, Biosafety Programs, prior to the transfer. It is the responsibility of the Principal Investigator transferring the Select Toxin to perform due diligence and to keep records for three years. Contact the UW Select Agent Program at uwsa@uw.edu for questions.

1. Abrin
2. Botulinum neurotoxins
3. Short, paralytic alpha conotoxins
4. Diacetoxyscirpenol (DAS)
5. Ricin
6. Saxitoxin
7. Staphylococcal Enterotoxins (Subtypes A, B, C, D, and E)
8. T2 toxin
9. Tetrodotoxin

X. RESOURCES

CONTACTS:

- **Work with toxins of biological origin:** EH&S Research and Occupational Safety, 206-221-7770, ehsbio@uw.edu https://www.ehs.washington.edu/staff/ehs-research-and-occupational-safety
- **Spills:** EH&S Spill Advice, 206-543-0467 https://www.ehs.washington.edu/staff/ehs-spill-advice
- **Waste collection and disposal information** EH&S Environmental Programs, 206-616-5835 or https://www.ehs.washington.edu/chemical/hazardous-chemical-waste-disposal
- **For mixed waste (i.e. toxin waste mixed with radioactive waste):** EH&S Radiation Safety, 206-543-0463, radsaf@uw.edu or https://www.ehs.washington.edu/staff/ehs-radiation-safety

FORMS:

- EH&S Online Chemical Collection Request: https://depts.washington.edu/ehas/pubcookie/prod/mychemwaste/index.php
- EH&S Biological Toxin SOP template: https://www.ehs.washington.edu/resource/biological-toxin-sop-550
- Diphtheria Toxin EH&S SOP template: https://www.ehs.washington.edu/resource/diphtheria-toxin-sop-547

- UW Select Toxin Transfer Due Diligence Form: https://www.ehs.washington.edu/resource/uw-select-toxin-transfer-due-diligence-form-66
- Biological Toxin Checklist: https://www.ehs.washington.edu/resource/biological-toxin-checklist-729

REFERENCE:

- Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition (December 2009). Centers for Disease Control and Prevention. Section VIII-G Toxin Agents, Appendix F: Select Agents and Toxins, and Appendix I: Guidelines for Work with Toxins of Biological Origin. https://www.cdc.gov/biosafety/publications/bmbl5/