

Exempt Select Agent Toxins

I. Harvard University Policy

This document establishes guidelines and policies for the possession, use, and disposal of select agent toxins that are exempt from the CDC and USDA Select Agent regulation. Select Agent Toxins are biological agents or toxins deemed threatening to the public, animal or plant health, or to animal and plant products. A laboratory facility that possesses more than the amount specified (<http://www.cdc.gov/od/sap/sap/toxinamt.htm>) for each toxin must register with the CDC/USDA, and fulfill all federal requirements in (1) 42 CFR Part 73, *CDC Interim Final Rule for Possession, Use, and Transfer of Select Agents and Toxins* (http://www.cdc.gov/od/sap/pdfs/42_cfr_73_final_rule.pdf), (2) 7 CFR Part 331, and (3) 9 CFR Part 121, *USDA Interim Final Rule for Possession, Use, and Transfer of Biological Agents and Toxins* (http://www.aphis.usda.gov/programs/ag_selectagent/FinalRule3-18-05.pdf). Laboratories having less than the stated amount are exempt from federal requirements, but must still adhere to the following policies.

This document serves as a guide for laboratories that possess and use exempt quantities of select agent toxins. Review this document and supply the information required in order to make it specific to your facility. Facilities that possess and use these materials should follow procedures, use appropriate containment equipment, and wear personal protection appropriate for the hazard posed by these materials. The CDC/NIH manual, *Biosafety in Microbiological and Biomedical Laboratories*, provides additional guidance for laboratories handling toxins. The manual is available at www.cdc.gov/od/ohs/biosfty/bmb14/bmb14toc.htm.

Laboratory personnel must call the Environmental Health & Safety (EHS) Department at 2-1720 (Longwood/Southborough Campus) or 5-2060 (Cambridge/Allston Campus) before possessing select agent toxins. EHS will help laboratories assess any hazard associated with an experimental procedure.

Registration with COMS: The laboratory must register ownership of the toxin(s) with the Committee for Microbiological Safety (COMS) prior to transfer and start of work. A registration form can be obtained through <http://www.hms.harvard.edu/orsp/coms/>.

Destroy of Toxin in the Laboratory: Before disposal, destroy Select Agent Biological Toxins by using appropriate chemical or physical agents (i.e. sodium hypochlorite and / or sodium hydroxide solution or by autoclave). Table 1 shows some chemicals used to deactivate toxins. The following procedures are recommended when destroying toxins:

1. Destruction Procedure

- Alert EHS at 2-1720 (Longwood/ Southborough) or 5-2060 (Cambridge/Allston).
- Use appropriate personal protective equipment.
- Perform the work inside a chemical fume hood. Ensure that the fume hood sash is at the appropriate working height.
- Prepare a fresh solution of chemical deactivating agent shown in Table 1.
- Line the work area with plastic-backed absorbent pads (Chux™).
- A toxin that is in powder form should be dissolved in solution, through the septum, without opening the container.
- For a toxin already in solution, open the container and place it in a secondary plastic container with a sealable lid.
- Carefully add the same amount of the chemical deactivating agent to the toxin.
- Allow at least 30 minutes for the chemical agent to deactivate the toxin.
- Replace the cap on the primary toxin container.
- Place and secure the lid on the secondary container.
- Label and dispose of the container as hazardous chemical waste.

2. Recordkeeping and Documentation

Destruction of the toxins should be properly documented and recorded. The following information should be obtained by the laboratory upon destruction of the toxin, and kept in the PI's records for five years.

- Name of toxin
- Copy of MSDS
- Manufacturer, if available
- Quantity
- Document procedure followed for destruction
- Name of person who destroyed the toxin
- Date and time of destruction
- Location of destruction
- Name(s) of witness(es)

VI. Personal Protective Equipment

At minimum, personal protective equipment for handling of toxins should include the following.

Eye protection: Safety glasses or chemical-splash goggles must be worn at all times when handling toxins. Ordinary prescription glasses do not provide adequate protection. Adequate safety glasses must meet the requirements of the "Practice of Occupational and Educational Eye and Face Protection" (ANSI Z.87.1 2003) and must be equipped with side shields and a brow guard. Safety glasses do not provide protection from splashes; chemical-splash goggles do.

Face shield: An optional face shield may be worn in addition to chemical-splash goggles when the potential for splashing exists.

Gloves:

1. Appropriate gloves shall be worn when handling toxins or toxic solutions.
2. Many chemicals may permeate gloves after a period of time. The selection of glove materials should be made according to the toxins and chemicals used in the procedures.
3. Gloves that protect against the generation of static charges are preferred, especially when handling dry or powdered forms of toxins.
4. Double-gloving with different types and colors of gloves is recommended for best protection.
5. Gloves are for splash protection, not immersion protection.

Respirator: Engineering controls, described in section III of the Chemical Hygiene Plan, are primary controls or safeguards. A NIOSH-approved respirator may be required if adequate engineering controls are not available or cannot contain aerosols generated by a specific operation, under select conditions, such as spill cleanup and decontamination. Please remember: medical evaluation, training, and fit-testing are required before wearing a respirator. Call EHS 2-1720 (Longwood/Southborough) or 5-2060 (Cambridge/Allston) for technical assistance before using a respirator.

Protective clothing: A traditional cotton-polyester white lab coat may not provide adequate protection against chemicals. Wear an impervious PE or PVC apron, lab coat, or smock when the possibility of skin contact exists.

VII. Emergency Procedures

Follow these steps when an emergency involving toxins occurs. Circumstances may include spills, fire and evacuation, personal exposure or injury, and power and ventilation failure.

Spill: Alert EHS to a spill. Wear disposable gloves, protective clothing, shoe coverings, safety glasses or chemical-splash goggles. Avoid raising dust when cleaning up. Ventilate the area and wash spill site after material pickup is complete. Wash contaminated clothing before reuse. Refer to an MSDS for proper cleanup measures.

Fire & Evacuation: Refer to the emergency flip chart titled *EHS Procedures and Response Guidelines*, and follow the standard procedure and the evacuation plan.

Personal Injury/Exposure, First Aid, & Medical Emergency:

A note to physicians: There are no known antidotes for tetrodotoxins, saxitoxins, and conotoxins, abrin, ricin, *clostridium perfringens* epsilon toxins, and T-2 toxin. Palliative treatment only. Artificial respiration to support breathing. Give oxygen, if breathing is difficult.

Refer to the emergency flip chart titled *EHS Procedures and Response Guidelines*, and follow the procedure for skin and eye contact. If a toxin is inhaled, move the victim to an area of fresh air. Seek medical aid immediately.

Power/Ventilation Failure:

1. Stop the work.
2. Secure and cover the toxin.
3. Whenever possible, store the toxin(s) in a secure storage location or chemical fume hood.
4. Lower the hood's sash completely.
5. Post an alert or warning on the sash.

Table 1. Inactivation Procedures for Select Agent Toxins

Toxins	Autoclave*	Chemical Inactivation allow at least 30 mins for the chemicals to deactivate the toxin				Comments
	1 hr at 121°C liquid cycle slow exhaust	2.5% NaOCl + 0.25N NaOH	2.5% NaOCl	1.0% NaOCl	0.1% NaOCl	
Abrin	Yes	N/A	N/A	N/A	N/A	
Botulinum neurotoxins	Yes	Yes	Yes	Yes	Yes	
<i>Clostridium perfringens</i> epsilon toxin	Yes	N/A	N/A	N/A	N/A	<i>*Methods of decontamination for the epsilon toxin do not appear to have been published.</i>
Conotoxins	No	N/A	N/A	N/A	N/A	Inactivated by reactive disinfectants such as glutaraldehyde and formaldehyde.
Diacetoxyscirpenol	No	Yes	Yes			
Ricin	Yes	Yes	Yes	Yes	Yes	
Saxitoxin	No	Yes	Yes	Yes	Yes	
Shigatoxin and Shiga-like ribosome inactivating proteins	Yes	Yes	Yes	Yes	Yes	Inactivated by oxidizing agents such as bleach and reactive sterilants such as glutaraldehyde.
Staphylococcal enterotoxins	Yes	Yes	Yes	Yes		
Tetrodotoxin	No	Yes	Yes	Yes	No	
T-2 toxin	No	Yes	No	No	No	The trichothecenes are very stable and resist heat-and ultraviolet-light-induced inactivation.

* Autoclaving can be effective against protein toxins (ricin, botulinum toxin, and SEB), but not with the low molecular toxins (mycotoxins, marine and venoms).

VIII. Laboratory Handling

General handling:

1. Since the chemical, physical, and toxicological properties have not been thoroughly investigated, use extreme caution when handling these toxins.
2. Any area where toxins are manipulated should be labeled as an OSHA "Designated Area." This includes all chemical fume hoods, bench tops, insulated areas, or the entire laboratory.
3. Avoid the use of ductless hoods or biosafety cabinets.
4. Consider class II, type B2, biosafety cabinets or chemical fume hoods for all work requiring manipulation of dry powder that has the potential for aerosolization. Sealed vials containing dry powder should be reconstituted with an appropriate liquid under original sealed conditions.
5. Concentrated stock solutions of the toxins may be used in a chemical fume hood. Consideration should be given to filtration of exhaust, location of exhaust, concentration of potential discharge.
6. Stock solutions should be stored in a well-marked area, away from public access. Individual containers should be labeled with the name, concentration and date of preparation. Consider storing stocks in secondary containment vessels for added safety.
7. Wear appropriate PPE, as recommended.
8. Wash hands thoroughly after handling, and avoid prolonged or repeated exposure to, toxins.

Waste handling and disposal:

1. Inactivation/Decontamination:
 - 1.1. Inactivation procedures for the toxins can be found in Table. 1.

Chemical Hygiene Plan: A sample SOP that fulfills Part III requirements

- 1.2. Exposure for 30 minutes to 1.0% sodium hypochlorite solution is an effective procedure for laboratory (utensils, glassware, working solutions, instruments, animal cages, working area and equipment) inactivation of saxitoxin, tetrodotoxin, ricin, botulinum toxin, or staphylococcal enterotoxins (SEB).
 - 1.3. For T-2 mycotoxin, it is recommended that, for complete inactivation, all liquid samples, accidental spills, and nonburnable waste be soaked in 2.5% sodium hypochlorite with 0.25N sodium hydroxide for 4 hours; expose cages and bedding from animals exposed to T-2 mycotoxin to 0.25% sodium hypochlorite and 0.025N sodium hydroxide for 4 hours.
2. Disposal:
- 2.1. Liquid waste: toxin solution residuals should be inactivated before disposal. Inactivated toxin effluent should be placed within a clearly labeled container in a chemical fume hood and disposed of as hazardous chemical waste.
 - 2.2. Solid waste: If the used gloves, paper towels, and other disposable supplies, such as pipet tips, contain residual toxin, discard them in a biohazard bag. Seal and place the biohazard bag in a biological burn box. Used needles should be discarded in a biohazard sharps container.
 - 2.3. Disposal of any Select Agent must be in accordance with the interim final rules on Select Agents and Toxins. Please call the EHS Department at 5-2060 (Cambridge/Allston) or at 2-1720 (Longwood/Southborough) for assistance in this matter.

IX. Common laboratory procedures involving select agent biological toxins

Develop and follow experimental procedures for all select agent biological toxins. These procedures shall be a component of the laboratory Chemical Hygiene Plan, will be self-reviewed and, if necessary, updated annually. A complete Chemical Hygiene Plan is available through *EHS, Industrial Hygiene, Chemical Hygiene/Lab Safety, Manuals – Chemical Hygiene Plan* (<http://www.uos.harvard.edu/ehs/ih/labsafety.shtml>). A sample procedure for preparing stock solutions of select agent biological toxins is provided below.

Preparation of Stock Solutions of Tetrodotoxin

1. Prepare solution(s) inside a chemical fume hood or a Class II, type B2, biosafety cabinet.
2. Dissolve 1 mg of TTX, along with 1 mg of citrate, in 3.1 mL of water to make 1mL stock solution.
3. Label individual containers of stock solution with the name, concentration, and the date of preparation of the active ingredient.
4. Stock solution is stored for several months at –20°C, in a well-marked area, away from public access.
5. Store stocks in secondary containment vessels for added safety.

Laboratory-specific Procedures

- Refer to equivalent procedures if they already exist in a lab manual.
- Specify the name of the reference manual.