



Bio Basics Fact Sheet: Guidelines for Work with Toxins of Biological Origin

Introduction:

- a. Biological toxins (BTs) are products of plants, animals, microorganisms (including, but not limited to, bacteria, viruses, fungi, or protozoa), or infectious substances, or recombinant or synthesized molecules.
- b. BTs are serious laboratory hazards that are highly toxic in minute quantities.
- c. BT routes of exposure are primarily inhalation, ingestion, and absorption (such as ocular, percutaneous, and injection). Skin absorption is also a potential hazard with some BTs.
- d. BTs do not pose a vapor hazard and do not have short-term exposure limits, ceiling limits or time-weighted average concentrations. They are different from well characterized chemical toxins and the unknown aspect of their properties must be considered in any risk assessment.
- e. Batches of BTs from the same source can vary widely in activity/toxicity.
- f. Risk assessment is key to developing and implementing an effective BT safety strategy.
- g. A 'zero level' toxin exposure philosophy will be the goal.

Risk Assessment:

Since each laboratory has its own unique facilities, and equipment for work with toxins, it is important to perform a risk assessment to determine exactly which physical and operational practices are essential to ensure effective risk management.

A risk assessment should include:

- Amount of toxin being worked with
- LD₅₀
- Probability of aerosol generation (powder or liquid form)
- Risks inherent to the procedure (inhalation of aerosols (intentionally and/or unintentionally created), auto-inoculation during animal procedures, static build-up when working with powders, etc.)
- Engineering controls
- Safety equipment availability and efficacy
- Personal protective clothing and equipment availability and efficacy
- Intoxication/lethality dose data
- Health effects data (acute and chronic)
- Availability of prophylaxis and/or treatment
- Training, experience of personnel, accident records
- Identification of specific hazards and mitigation of these before the commencement of work with BTs

General Safety Practices:

- Provide all laboratory personnel with training specific for the toxins being used

- Routine operations with dilute toxin solutions are to be conducted under BSL2 conditions with personal protective equipment and a BSC that is certified annually or comparable engineering controls as determined by risk assessment
- A standard operating procedure (SOP) for toxins is to be developed that covers all aspects of toxin work including:
 - i. Basic handling and experimental protocols
 - ii. Procurement
 - iii. Distribution
 - iv. Storage
 - v. Decontamination and detoxification
 - vi. Disposal

NOTE: SOP template is found at <http://www.dehs.umn.edu/PDFs/writingSOP.pdf>

- Maintain an accurate inventory
- Store toxin stocks in locked storage rooms, cabinets, or freezers
- Toxins are to be transported in spill and leak-proof secondary containers
- Preparation and manipulation of toxin stock solutions and primary containers of dry toxins including other high risk procedures is to be conducted in a biological safety cabinet (BSC) that is certified annually

NOTE: Approvals will not be granted for future for toxin work outside of a BSC except where a detailed risk assessment makes a convincing case that risks will be mitigated through the use of alternative safety procedures when manipulating the toxin. This assessment will include, at a minimum, the amount of toxin to be used, the probability of aerosol generation, the route of exposure and the LD50.

- Redundant HEPA filtration is required when conducting high risk operations/procedures with BTs such as:
 - i. Working with BTs in powdered form
 - ii. Manipulating BTs in ways that intentionally generate dust or aerosols
- Every effort must be made to work with less than 1/10th of one LD₅₀ dose of toxin
- Toxin laboratories should be maintained at a negative pressure compared to adjoining rooms, corridors or other areas
- Whenever possible, reconstitute entire vial of powdered toxin by injecting diluent through septum
- Two knowledgeable individuals should be present in the laboratory during toxin manipulation whenever high-risk procedures are performed
- Gloves are to be selected that do not generate static electricity and are impervious to the toxin and solvent
- Verify inward airflow of the biological safety cabinet before initiating work
- Post the entrance(s) to the room with a biohazard sign identifying the hazard as "Biologically-Derived Toxin" and indicate any special entry requirements when toxins are in use
- Decontaminate the exterior of the primary container and place in a clean secondary container before removing toxin containers from the BSC. Transport toxins in leak/spill-proof secondary containers.

Toxin Inactivation (*Revised on January, 2017*):

Table 1: Inactivation of proteinaceous biological toxins

Toxin (proteinaceous)	Autoclave 1hr @ 121°C	NaOCl (30 min)	NaOH (30 min)	NaOCl+NaOH (30 min)	Comments
Abrin ⁽⁴⁾	Yes	ND	ND	ND	176°F (80°C) most of the toxicity is lost in 30 minutes.
Botulinum neurotoxins ⁽¹⁾	Yes	>0.1%	>0.25N	ND	>0.1% NaOCl or >0.25N NaOH for 30 min is recommended for decontaminating work surfaces and spills.
Clostridium perfringens epsilon toxin ⁽²⁾	Yes	ND	ND	ND	Decontaminate with soap and water. The protein is heat-labile.

Toxin (proteinaceous)	Autoclave 1hr @ 121°C	NaOCl (30 min)	NaOH (30 min)	NaOCl+NaOH (30 min)	Comments
Staphylococcal enterotoxins ⁽¹⁾	Yes	>0.5%	>0.25N	ND	SEB is heat stable. SEB is inactivated with 0.5% NaOCl for 10-15 min.
Ricin ⁽¹⁾	Yes	>1.0%	ND	>0.1%+0.25N	1.0% NaOCl is effective for decontaminating surfaces, equipment, animal cages, or small spills.
Shigatoxin and Shiga-like ribosome inactivating proteins ⁽³⁾	Yes	0.5%	ND	2.5%+0.25N	0.5% NaOCl is recommended for spills.
Diphtheria toxin ⁽³⁾	Yes	0.5%	ND	ND	0.5% NaOCl is recommended for spills.
Tetanus Toxin ⁽³⁾	Yes	0.5%	ND	ND	0.5% NaOCl is recommended for spills.
Pertussis toxin ⁽³⁾	Yes	0.5%	ND	ND	0.5% NaOCl is recommended for spills.
Cholera toxin ⁽³⁾	Yes	0.5%	ND	ND	0.5% NaOCl is recommended for spills.
Anthrax Lethal Toxin (PA, LE) ⁽³⁾	Yes	>=0.5%	ND	ND	0.5% NaOCl is recommended for spills.

Table 2: Inactivation of low molecular weight toxins

Toxin (low molecular weight)	Autoclave 1hr @ 121°C	NaOCl (30 min)	NaOH (30 min)	NaOCl+NaOH (30 min)	Comments
Tetrodotoxin ⁽¹⁾	No	>=0.5%	ND	0.25%+0.25N	1.0% NaOCl for 30 min contact time is effective for decontaminating nonburnable waste, equipment, animal cages, work area, and spills. The burnable waste from Tetrodotoxin should be disposed of in a yellow waste bag*.
Saxitoxin ⁽¹⁾	No	>=0.1%	ND	0.25%+0.25N	1.0% NaOCl for 30 min contact time is effective for decontaminating nonburnable waste, equipment, animal cages, work area, and spills. The burnable waste from Saxitoxin should be disposed of in a yellow waste bag*.
T-2 mycotoxin ⁽¹⁾	No	>=2.5%	ND	0.25%+0.25N	For T-2 mycotoxin & brevetoxin, liquid samples, spills, and nonburnable waste should be soaked in 2.5% NaOCl with 0.25N NaOH for 4 hrs. Cage and animal bedding should be treated with 0.25% NaOCl and 0.025N NaOH for 4 hrs. The burnable waste should be disposed of in a yellow waste bag*.
Diacetoxyscirpenol (DAS), Deoxinolenol (DON), Zearalenone (ZEA) ⁽¹⁾	No	>=2.5%	ND	0.25%+0.25N	Dispose solid items contaminated with DAS, DON, and ZEA in a yellow waste bag. 2.5% NaOCl is recommended for spills.
Microcystin ⁽¹⁾	No	>=0.5%	ND	0.25%+0.25N	See note 3.
Palytoxin ⁽¹⁾	No	>=0.1%	ND	0.25%+0.25N	See note 3.
Brevetoxin ⁽¹⁾	No	>=2.5%	ND	0.25%+0.25N	2.5% NaOCl is recommended for spills.
Conotoxin ⁽⁵⁾		0.5%			Conotoxins containing S=S bonds (e.g. omega-conotoxin) can also be inactivated by 1% v/v glutaraldehyde or formaldehyde (30-min contact time and sewer). Decontaminate work surfaces with 10% bleach. Dispose of used pipette tips in 10% bleach or 1% v/v glutaraldehyde. Dry waste (e.g. gloves) can be autoclaved at 121°C for 1hr at 18 psi.

*According to BMBL 5th edition, [Appendix I—Guidelines for Work with Toxins of Biological Origin](#), autoclaving with 17 lb

pressure (121-132° 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° F). Since the red biohazard waste bags are autoclaved by the waste contractor, LMW toxins should be disposed of in a yellow waste bag.

- Notes:**
1. ND indicates not determined
 2. Household bleach generally is a 5.25% solution of sodium hypochlorite (NaOCl). A dilution of 1 part household bleach to 9 parts liquid is a 0.525% solution.
 3. All low molecular weight 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. Alkalinity, rather than oxidizing ability, is the inactivation factor. The burnable waste from LMW toxins should be disposed of in a yellow waste bag.
 4. The number following each toxin corresponds to the number in the following section **References for toxin inactivation methods.**

References for toxin inactivation methods:

1. CDC/NIH BMBL 5th edition, [Appendix I—Guidelines for Work with Toxins of Biological Origin](#)
2. [Fact sheets on Chemical and Biological Warfare Agents](#)
3. List Biological Laboratories, Inc. [MSDS](#)
4. CDC Abrin [Emergency Response Card](#)
5. Manual of Security Sensitive Microbes and Toxins, Part IV Microbes and Toxins Affecting Human and Animals: Toxins, [41.Conotoxins](#), page 29

Spill Clean-Up and Accidental Exposure:

Use an effective inactivating agent for a specific toxin (see table 1 & 2 above). Follow decontamination procedures in the [Biohazards and Toxin Decontamination & Spill Clean-up](#) fact sheet to clean-up spill. If spill results in a hazard exposure, follow **Accidental Exposure Response** in the fact sheet.

Regulated Toxins

- Toxin users must consult the following to determine whether the type and amount of toxin used falls under the Select Agent regulations:
 - 1) SA list: <http://www.selectagents.gov/Select%20Agents%20and%20Toxins%20List.html>
 - 2) Permissible Toxin Amounts: <http://www.selectagents.gov/Permissible%20Toxin%20Amounts.html>
- If the Select Agent Regulations apply in your case, please contact DEHS Biosafety at 612-626-6002.

Institutional Biosafety Committee Oversight:

All biologically-derived toxin work must be approved by the Institutional Biosafety Committee (IBC) before work is started. Approved protocols are effective for three years with annual review.

- Submit toxin forms by downloading from <http://www.research.umn.edu/ibc/forms.html>

Other Useful Information:

- Toxin guidelines in Appendix I, *Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition*, http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_appendixI.pdf