Safety and Health Considerations for Conducting Work with Biological Toxins

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Introduction

The biosafety-chemical safety “gray zone” when working with toxins is the focus of this article. Numerous guidelines and recommendations exist describing procedures, personal protective equipment, containment equipment, and engineering controls to promote safety and health when working with toxic chemicals and infectious agents (Miller, 1993; Richmond & McKinney, 1999). However, limited attention has been given to work with nonreplicating toxic biological materials. The authors’ goal is to provide an overview of pertinent safety and health information for work with toxins, while highlighting the more unique issues associated with this type of work.

Material Safety Data Sheets (MSDS), Threshold Limit Values (TLV), Time Weighted Averages (TWA), and Biological Exposure Indices (BEI) (see Definitions) have been developed to help inform individuals about the potential risks associated with specific toxic chemicals. These documents and others assist safety professionals and researchers in selecting engineering controls and protective clothing/equipment that will reduce the risk of exposure.

Work with toxic chemicals is associated with the risk of exposure to gases, vapors, mists, or particulates. Quantifying the potential hazard to workers is traditionally expressed as a TWA (for example, ppm or mg of chemical per cubic meter of air over a unit time). Biological toxins are unique and stand separate from toxic chemical classification schemes because toxins are highly toxic in very minute quantities, they typically present an airborne particulate hazard to workers, but they are not associated with production of vapors or gases. In addition, no short-term exposure limits, ceiling limits, or time-weighted average concentrations have been established for safe work with toxins (as is the case with toxic chemicals), and currently no commercially available real time detectors to monitor worker exposure to toxins exist. Also, in many cases toxicological data on toxins are very limited, especially for chronic exposure with sublethal (or subsymptomatic) concentrations. In light of the above, the authors have employed a “zero level” toxin exposure philosophy in formulation of the information that follows.

Due to the unique properties of biological toxins, their safe handling in the workplace is dependent upon:
1. A management chain committed to providing support and resources to the safety and health program
2. A thorough understanding of the toxin and assessment of risk
3. Adherence to safety practices and principles by the workforce.

This article does not provide a compilation of highly detailed information on each toxin; rather, it provides brief examples to illustrate various points, which hopefully will assist in developing appropriate safety protocols and programs for different facilities.

The remainder of this article is offered to assist both researchers and safety/health professionals in planning toxin operations and is organized as follows:
- Definitions
- The Nature of Biological Toxins
- Risk Assessment
A Note on Regulatory Requirements
Facility Recommendations for Toxin Work
Engineering Controls for Risk Mitigation
Protective Clothing and Equipment
Recommendations for Personnel Working with Toxins
Good Work Practices with Toxins
Decontamination Recommendations
Emergency Response Recommendations
Security Considerations for Toxin Laboratories

A final introductory note on the role of leadership for the safety professional formulating a successful safety program: Leadership with the knowledge and assistance in implementation of the safety professional creates the safety system. The safety system includes all aspects of the work environment (e.g., hiring practices, training, awards, facilities, safety equipment and procedures, oversight processes). Once the safety system is created, the product of that system becomes predetermined. This is true for both successful and unsuccessful safety programs. The immediate and long-term products of the safety system encompass providing a safe environment for workers, while facilitating their ability to perform their duties with minimal impediments. The safety system is dynamic and requires refinements over time. Once the system is in place significant changes should not occur without modifying the system. If the safety program results or products are consistently unacceptable, a system-level change is needed.

While occasionally problems may be traced attributed to an individual, a comprehensive functional safety system should prevent recurrent problems. Problems are more likely a symptom of an overall system shortcoming, rather than just one weak link in the chain (i.e., "a poor employee"). The refinements or changes needed may be as overarching as redefining chains of command, reporting, and responsibilities, or as restricted as instituting a particular safety training program. It is leadership's challenge, guided by informed counsel from safety professionals, to establish, monitor, and actively participate in the institution's safety and health program.

While a system-level approach is desirable for any program, it is required for safety programs because safety programs rely on each laboratory worker's mindset to achieve a safe work environment. While engineering controls, immunizations, etc. are valuable ad-

juncts, the cornerstone of a successful safety program is the commitment of each worker to safe work practices. The authors believe that achieving such a commitment from each worker requires a system-level approach, as promulgated by leadership. Leadership must be an active advocate and participant in the safety program.

Definitions

BEI. Biological Exposure Indices. The measurement of chemical contaminants in the workplace as determined by the concentration of the chemical in biological samples. For example, the amount of carbon monoxide permitted in the workplace can be presented as parts per million in human blood tissue.

Dalton (d). The measure of atomic mass, also referred to as atomic mass unit. Toxins range in mass from several hundred daltons up to 150,000 or more daltons.

HEPA filter. A high-efficiency particulate air filter, typically capable of filtering 99.97% of 0.3 micron mono-dispersed airborne particulates.

HVAC. The heating, ventilation, and air conditioning systems in a building. This term includes the exhaust air and supply air systems for laboratory ventilation.

LD₉₀. The dose to an animal or human in terms of micrograms/kg body weight, which is lethal in 50% of the cases.

LFPm. Linear feet of air per minute. A measurement of air velocity, such as at the opening of a fume hood.

MSDS. Material Safety Data Sheet. The format used by the chemical industry to alert users of the toxicity, flammability, and other hazards associated with a particular chemical. MSDSs are also developed by some toxin suppliers.

NIOSH. National Institute for Occupational Safety and Health, the U.S. proponent for respirators.

PPC&E. Personal protective clothing and equipment.

PPM. Parts per million.

SEB. Staphylococcal enterotoxin B.

Toxin. For the purpose of this article, toxin refers to biological toxin. Biological toxins are any toxic substance that can be produced by microorganisms, animals, and plants.
TLV. Threshold Limit Values. Refers to the airborne concentration of a chemical (generally refers to an exposure level that a worker can experience day after day without adverse health effects). The TLV can be expressed in terms of time-weighted average (TLV-TWA), short-term exposure limit (TLV-STEL), or the ceiling concentration (TWA-C).

TWA. Time Weighted Average. Also known as the TLV-TWA, this concentration of chemical is weighted over an 8-hour workday and 40-hour work week. The TWA is the concentration that "nearly all workers may be repeatedly exposed, day after day, without adverse effect" (ACGIH, 1999).

The Nature of Biological Toxins

Definition

Traditionally, biological toxins have been described as any toxic substance that can be produced by microorganisms, animals, and plants. Examples include botulinum toxins (A-G), tetanus toxin, and staphylococcal enterotoxins (A-F), which are produced by bacteria; tetrodotoxin, batrachotoxin, and ciguatoxin, which are produced by animals; and ricin toxin, trichothecene mycotoxins, and abrin, which are produced by plants (Whalley, 1990). Toxins are not living organisms, nor do they fall neatly into the category of the "classic" organic chemicals.

Classification

Classification of toxins as biological materials rather than toxic chemicals can be confusing when one considers that some biological toxins are now produced in laboratories in nonnative hosts using cloning and other molecular, chemical, and biochemical techniques. Additionally, two prominent toxins of biological origin—saxitoxin and ricin toxin—have been added to the international Chemical Weapons Convention (CWC) list of Schedule 1 chemicals (CWC, 1993). Because toxins are not living organisms, work with them is not assigned a biosafety level (Richmond & McKinney, 1999); however, requirements for the possession and transfer of certain biological toxins are described in the Select Agent Transfer Program (Title 42 CFR Part 72.6 1996).

The paradox that biological toxins are not "classic" organic chemicals yet not infectious agents has placed them in a biosafety-chemical safety gray zone with few resources specifically addressing protocols for safe work (Title 32, CFR Part 626, 1991; Title 32 CFR Part 627, 1992). As these are nonreplicative materials, the authors believe that they should not be assigned placement solely using the traditional BSL-1 through BSL-4 categorization (unless the microorganism of origin is involved, at which point the microorganism is assigned a biosafety level).

Since they are not replicating agents, it is appealing to classify toxins as standard chemicals and consider developing TWAs for them. However, most biological toxins are inherently different from toxic chemicals in several ways. Unlike standardized manufactured chemicals, batches of toxin from the same source can vary widely in the actual quantity of active toxin produced. As many facilities may produce their own materials, and numerous suppliers exist, it becomes impractical to suggest developing a safety standard based on quantity of material rather than active units. Measurements of activity units are often expressed in the context of a specific assay, with different laboratories using different assays. These assays do not always correlate with each other to give a universal standard measurement, adding to the difficulty of trying to develop a TWA or standard approach to safety when handling biological toxins.

Unlike the case for toxic chemicals where supportive treatment is the only avenue following exposure, prophylactic vaccines and antitoxins also exist for some biological toxins (along with several under development). While many toxic chemicals are associated with carcinogenic, teratogenic, and chronic effects, most biological toxins manifest acute effects, though a few produce long-term effects (such as Pfiesteria toxin[s]).

In terms of differences in routes of exposure, biological toxins, unlike toxic chemicals, do not pose a vapor hazard and few are dermally active. The threats are primarily inhalation, ingestion, and injection. For mycotoxins, Pfiesteria toxins, and some other toxins, skin absorption is also a potential hazard.

Finally, the development of TWA-based safety parameters for work with biological toxins is complicated by the high variability in response to a given biological toxin by different species, interanimal physiologic
variation, different routes of exposure, and purity or activity of the preparation. Assuming linearity and extrapolation to humans from models generated by dose-response data in other species is simply not a valid approach in developing guidelines for safe work with biological toxins.

A rational approach would involve combining the most efficacious and applicable safety practices from both the chemical and microbiological worlds. To do this one must begin to understand the nature of biological toxins.

**Characteristics**

Biological toxins are nonreplicative, noninfectious materials, which are not passed among individuals or to other potential hosts in a communicable manner. They are not transmitted by vectors such as insects or host animals. They may be transferred to individuals by manual contamination (for example, food stored in a laboratory refrigerator may become contaminated if splashed by solutions containing toxins). Toxins do not persist for long periods in the environment, lie dormant and reemerge, concentrate in any given environmental matrix, or pose a vapor hazard. Their physical characteristics are as varied as their natural sources ranging in molecular weight from approximately 300 daltons (saxitoxin and tetrodotoxin) to 150,000 daltons (botulinum toxin A and tetanus toxin).

Most biological toxins are proteinaceous; the few exceptions include trichothecene mycotoxins produced by fungi. As a general rule, most biological toxins such as botulinum toxins and staphylococcal enterotoxins are fairly stable in impure or undiluted forms (Facklam, 1983; Robins, 1973), though some like ricin are very stable in a variety of otherwise harsh conditions (Facklam, 1983). Purified biological toxins are nonvolatile, colorless, odorless, and tasteless, and most are not dermally active (exceptions include some trichothecene mycotoxins and Pfiesteria toxin[s]).

**Toxicological Aspects**

A wide variety of sources are available to researchers and risk management personnel for information on the toxicology of toxins (also known as “toxinology”). This article can not adequately cover the vast amount of information on this subject; however, this information can provide a roadmap to the available literature. The following sources are provided (also see Reference section at the end of this article):

- **Material Safety Data Sheets (MSDS):** MSDSs vary widely in quality and comprehensiveness. For the most part, toxin MSDSs offered by major chemical supply houses are excellent and provide both the researcher and safety/health professional a synopsis of toxicity, route of entry, symptoms, first aid, and, in some cases, special information for medical practitioners. Note that MSDSs produced by the chemical manufacturing community are often based on handling production quantities of materials and some MSDS sections may not always apply to laboratory situations.

- **Army Field Manual 3-9, Chapter 4:** A very comprehensive source book of animal and human lethality of a wide variety of toxins, this information covers (in typical military fashion) a very short description of the various toxins, route of entry, rate of action, symptoms, treatment, toxicity, and decontamination.

- **Medical Management of Biological Casualties, USAMRIID, 3rd edition. Itzen, et al., 1998:** Although this handbook is designed for medical treatment in military scenarios, it does contain very useful information on toxins. Of special note is the information written for the medical practitioner on diagnosis and medical management of intoxicated personnel.

- **Handbook of Toxicology** (Shier & Mebs,1990): An excellent source for information for researchers and medical practitioners, this handbook is organized around toxins' mechanisms of action at the biochemical, physiological, and pathological level.

- **Handbook of Natural Toxins** (Hardedge & Tu,1988): This eight-volume handbook is a valuable resource for both the researcher and safety professional. The set includes separate volumes on plant/fungal toxins, insect poisons, marine toxins/venoms, bacterial toxins, and reptile/amphibian venoms.

**Internet Resources**

ABSA Home Page (Links to many biosafety re-
Sources:
http://www.absa.org/
CDC/NIH, Biosafety in Microbiological and Biomedical Labs, 4th ed.: http://www.cdc.gov/od/ohs/biosfty/bml/bml-1.htm

Routes of Exposure

Biological toxins' routes of exposure, which pose risks to the worker, are similar to those associated with infectious agents. Primary routes are through ingestion, inhalation, absorption (such as ocular, percutaneous, and injection). A more unique route of entry for toxins is by cutaneous absorption, as toxins may be solubilized in substances which act as carriers across the skin (such as dimethyl sulfoxide [DMSO]). Adhering to good microbiological practices and the use of correct PPC&E can minimize if not eliminate these risks. Certain procedures create more hazards than others, some of which can be avoided. The section entitled "Good Work Practices with Toxins" below provides several examples of safety procedures to help minimize hazards associated with specific laboratory procedures.

Administratively, in addition to providing training and guidance through operating procedures, information directly relevant to each toxin can be generated and provided to the workforce. MSDSs have proven their value as useful tools for safety professionals and researchers by identifying health hazards, physical/chemical properties, methods of neutralization, and other information pertinent to a particular chemical, but MSDSs may not be available for some biological toxins (particularly newly identified ones). Therefore, a safety and health program should include the development of an information source for biological toxins similar in scope to the MSDS. Figure 1 offers a sample "Biological Toxin Summary Sheet."

Risk Assessment

Risk assessment is one of the cornerstones in the development and implementation of a viable toxin safety and health program. The risk assessment process is especially important when dealing with biological toxins, since their unique nature often requires nontraditional and creative solutions to reduce hazards. To be successful, a risk assessment should be a joint venture between researchers and safety/health professionals.

In the laboratory or toxin work area, the risk assessment primarily encompasses quantitative information and includes:

- Risks inherent to the procedure (auto inoculation during animal protocols, inhalation of intentionally or unintentionally created aerosols, static build-up when working with powders).
- Probability of generating an aerosol
- Amount of toxin being worked with
- Availability of successful treatment
- Availability of vaccines or antitoxins
- Training and experience of personnel
- Intoxication/lethality dose data
- Health effects of exposure (acute and chronic)
- Accident records (Is there a trend indicating increased probability of exposure?)
- Engineering controls
- Safety equipment availability and efficacy
- PPC&E efficacy and availability
- Maximum credible event (Define a worst case incident and how to minimize the adverse results.)

Another helpful tool in developing a risk assessment is for risk management personnel and researchers to hold a 1-hour "what-if" hazard analysis session. This "what-if" session is an opportunity to brainstorm the possible failure scenarios when working with toxins. Table 1 shows two examples of "what-if" failure scenarios.

The risk assessment is then communicated to workers, management, first responders, and other parties impacted by the work. In some instances the local community may also be involved. In these situations the assessment must also consider intangible or per-
ceived risk (qualitative risks), often the root of the "not in my backyard" philosophy. These qualitative risks may be driven by lack of knowledge, fear of catastrophic events, devaluation of property value due to hazardous work in the facility, neighborhood releases/death, and feelings of disenfranchisement, to name a few. By identifying risk elements accurately, one can develop and implement policies and processes to eliminate the hazard or reduce the hazard to an acceptable level.

Communicating this information should help to allay fears and demonstrate to workers, management,

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**Figure 1**
Suggested Format for a "Biological Toxin Summary Sheet"

**Section 1—Agent**
- Name:
- Natural source:
- Source in facility: (such as: genetically engineered or modified)
- Characteristics: (such as: molecular weight, composition, resistance to denaturation, inactivation under normal storage conditions, immunological serotypes, etc.)
- Decontamination: (personnel/areas/equipment)

**Section 2—Health Hazards**
- Route of entry:
- Signs/symptoms of intoxication: (by route of entry)
- Toxicity dose data: (by route of entry, primate data preferable)

**Section 3—Medical**
- Diagnosis:
- Prophylaxis (immunization or antitoxin):
- Treatment:
- Decontamination: (personnel)
- Emergency first aid/self-aid:
- Accident/incident reporting protocol: (list of notification names, phone numbers and locations for treatment)
- Recommended protective clothing and equipment:

**Section 4—Decontamination**
- Susceptibility to decontaminants:
- Time for effective decon:
- Concentration of decon solution:
- Shelf-life of decontaminating solutions:
- Physical inactivation (time and other parameters such as temperature):

**Section 5—Safety Precautions**
- Procedures and practices (specific procedures such as handling liquids, syringes, powders, aerosols, etc.):
- Protective clothing and equipment:
- Containment equipment:

**Section 6—Facility Engineering**
- HVAC: (exhaust air requirements/supply air requirements)
- Showers/eyewash stations:
- HEPA filter recommendations:
Table 1
Example of a “What-If” Hazard Analysis

<table>
<thead>
<tr>
<th>HAZARD</th>
<th>CONSEQUENCE</th>
<th>HAZARD MITIGATION</th>
</tr>
</thead>
</table>
| Needle stick/sharps hazard     | Contaminated syringes or other sharps could introduce toxins through gloves into worker's skin. | 1. Minimize use of sharps.  
                                  |                                                                             | 2. Maintain adequate supply of approved sharps disposal containers.  
                                  |                                                                             | 3. Use syringes which resheath the needle.  
                                  |                                                                             | 4. Train operators in sharps precautions. |
| Spill of toxin outside of fume hood | • Possible dermal contact with toxin  
                                  | • Possible production of toxin aerosol, causing inhalation hazard  
                                  | • Possible spread of contamination to clean areas | 1. Operators have established and rehearsed spill procedures.  
                                  |                                                                             | 2. NIOSH-approved respirators are available.  
                                  |                                                                             | 3. Sufficient supply of appropriate decon is available.  
                                  |                                                                             | 4. Operators know and understand emergency procedures. |

and the community that the level of gain far outweighs the level of risk. There is a range of what can be perceived as a good or an adequate level of acceptable risk, but there is no unequivocal delineation for where acceptable ends and unacceptable begins. It is important to understand that different institutions and individuals will have different thresholds for risk acceptance, and that no matter how accurate the risk assessment and how exceptional the proposed mitigation plan, some people will not be satisfied. The ultimate goal is to ensure safety and health in the workplace, to protect personnel outside the facility, and to protect the environment.

A Note on Regulatory Requirements

Contractor Requirements for Biodefense (United States)

Any organization that performs “bio-defense” work with toxins for the U.S. government is subject to the requirements in Title 32 CFR parts 626 and 627. Although the regulation applies only to biodefense, it closely follows the recommendations found in the CDC/NIH publication, *Biosafety in Microbiological and Biomedical Laboratories* (1999). In addition, Title 32 CFR part 627 contains a section on toxin safety (Title 32 CFR 627.28 and 627.29). The regulation can be found on the Internet at: http://www.access.gpo.gov/nara/cfr/waisidx/32cfr627.html.

Government Requirements for Biodefense (United States)

The requirements described in the paragraph above also apply to all U.S. government laboratories working with toxins in the biodefense mission arena.

Shipping/Receiving Toxins and CDC Registration

The U.S. federal law cited in Title 42 CFR part 72.6, known as the Select Agent Transfer Program, applies to any work in the United States with certain microbiologicaals and the toxins listed below. Work with toxins regulated by federal law requires organizations to register with CDC prior to shipping or receiving these materials. Toxins that are currently regulated are:

- Abrin
- Aflatoxins
- Botulinum toxins
- Conotoxins
- Clostridium perfringens epsilon toxin
- Diacetoxyscirpenol
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- Ricin
- Saxitoxin

The regulation and information on registering an institution with CDC can be found at the following Internet site: http://www.cdc.gov/od/ohs/lrsat.htm

OSHA “Right to Know” Legislation

U.S. federal law requires employers to notify workers of all workplace hazards. For toxins, this should be accomplished by training new workers, along with posting MSDSs or Toxin Summary Sheets in the workplace.

Facility Recommendations for Toxin Work

The challenge when designing a facility or procuring engineering controls is to balance cost with the potential risk and available floor space. For example, it may cost much less to install biosafety cabinets (with installed HEPA filters) in a laboratory than to install fume hoods with HEPA filter banks downstream at the exhaust blower. Decisions on risk vs. cost vs. available floor space should be made during the risk assessment process when planning new facilities or modifying existing ones.

The authors recommend redundant HEPA filtration whenever working with powdered toxins or for operations which intentionally generate aerosols. A typical HEPA filter is 99.97% efficient when properly installed in an exhaust ventilation system. If the primary filter fails (due to air gaps in the filter seal, etc.), the secondary HEPA (in series) will ensure that toxins are not exhausted inadvertently into the environment. (Note: In some cases, the primary HEPA filter may be built into the biosafety cabinet with the secondary HEPA filter installed downstream near the exhaust system blower.) For work with toxins in solution, a single level of HEPA filtration is adequate providing the operation does not involve intentional aerosol production.

Exhaust ventilation stack heights and stack location should be carefully considered. The design should follow good industrial hygiene practices as described in the latest edition of the ACGIH Industrial Ventilation Handbook (ACGIH, 1998). Avoid weather caps on stacks because they interfere with the effective vertical discharge of exhaust air from the laboratory.

HEPA filters used for toxin work should be performance tested when installed and then tested annually by trained technicians using polydisperse dioctyl phthalate (DOP) or similar particulate simulant. The authors recommended the filter test protocol outlined in HEPA and ULPA Filters (Institute of Environmental Sciences, 1993).

Toxin laboratories should be maintained at a negative pressure compared to adjoining rooms, corridors, or other areas. Ideally, the airflow velocity from corridors/areas into a toxin lab is approximately 100 lpm measured in the open doorway to the lab (ANSI/ASHA, 1992). When measuring airflow velocity in the lab’s open doorway, make sure the flow is into the lab at all points in the doorway opening (can be verified visually with smoke tubes). Typical negative pressure for a well-designed toxin laboratory ranges from 0.005 to 0.05 inches of negative pressure (water gauge) in comparison to other areas.

Dual HEPA filters in series should have a differential pressure gauge before the filter bank and between HEPA filter banks. Periodic monitoring of the differential pressure gauge will alert operators to particulate “loading” of the first HEPA filter.

Toxin laboratories should use an exhaust ventilation system, which offers from 10 to 12 room air changes per hour. Higher room air change rates are sometimes cited in the literature (Mayer, 1995), but higher rates can increase the heating/air conditioning burden. The make-up air to rooms should be provided through the ceiling via a diffusion panel in order to minimize turbulence and eddy currents.

A good rule for designing toxin laboratory ventilation systems is to provide 10% more exhaust air than make-up air, thus ensuring a reasonable amount of negative pressure (and proper airflow) for the toxin work area. Air should always flow from corridors and other clean areas into the toxin work area and should be verified by smoke tests or differential pressure gauges.

An emergency shower, eye wash station, and hand-washing sink must be readily available to all locations where toxins are used. For work with toxins that can be absorbed through the eyes (such as mycotoxins), an
eyewash must be located in each laboratory room.

Use an emergency back-up power generator for critical operations such as conducting aerosol studies, work with lyophylized toxins, or pharmaceutical dispensing/filling operations. Deciding whether or not to procure expensive back-up power equipment should be based on the risk assessment. If the risk assessment shows a potential for personnel exposure due to a loss of power to the exhaust ventilation system, a back-up power generator should become a high priority.

Interlocked supply air and exhaust air fans are desirable so that a failure of the exhaust air fan will automatically activate the shut down of the supply air system. Without the interlock system, exhaust system failure could result in a room-positive pressure condition, thus potentially disseminating toxic material into corridors or other clean areas.

Exhaust ventilation systems should employ a visible and audible alarm for loss (or severe reduction) of exhaust air, such as due to an electrical power failure or an exhaust air blower failure. Alarms should be able to function even during a complete electrical power failure and at a predetermined level (such as below 75 fpm for fume hoods or below 0.25 inches negative pressure for gloveboxes).

Engineering Controls for Risk Mitigation

A number of engineering controls can be used successfully to manipulate toxins. Select a primary engineering based on factors such as toxin quantity, toxin form (powder vs. solution), relative toxicity of the toxin, and the nature of the toxin manipulation. Work with the dry, powdered form of toxins can present unique hazards due to air turbulence and electrostatic phenomena. The risk assessment process should include an evaluation of toxin manipulation procedures, and this information should be used to determine the right engineering control for the operation.

The authors recommend biosafety cabinets and gloveboxes for toxin operations; however, some very dilute/low-level toxin manipulations may present negligible risk to operators and may be suitable for bench top work without engineering controls. Again, the risk assessment process will help safety professionals and researchers make sound decisions on the need for engineering controls and the best type of engineering control for each biological toxin protocol.

Biological Safety Cabinets

A detailed description of the design, proper selection, use, and performance testing of biological safety cabinets (also known as biosafety cabinets) can be found in the CDC/NIH publication, Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets (Richmond & McKinney, 1995; Internet site: http://www.cdc.gov/od/ohs/biosfty/bsc/bsc.htm).

Biosafety cabinets, if properly chosen and maintained, are preferable to fume hoods when working with toxins. The authors recommend Class II, Type B2, or Class III biosafety cabinets for toxin work, since in both cases the exhaust air is filtered through a built-in HEPA filter which prevents the possible contamination of downstream duct work, exhaust ventilation equipment, and the environment. Note also that both Class II, Type B2, and Class III biosafety cabinets can also be used for work with small quantities of volatile toxic chemicals and radionuclides.

A Class II biosafety cabinet resembles a fume hood but has some significant differences. These include both HEPA-filtered exhaust air and HEPA-filtered supply air, thus also protecting the integrity of the toxins used in the biosafety cabinet.

A Class III biosafety cabinet resembles a glovebox, but as noted above contains exhaust and intake HEPA filters (intake HEPA protects the work surfaces). Class III biosafety cabinets should operate at approximately 0.50-inch negative pressure (water gauge) (Richmond & McKinney, 1995).

For some applications such as work with large quantities of toxins (especially if aerosol production is possible), one should consider hard-ducting biosafety cabinets to the room exhaust ventilation system. This ventilation system should direct exhaust air to the building exterior in a manner consistent with good industrial hygiene design. Examples of good exhaust air design include minimal use of ducted elbows, exhaust stacks located downstream of building air intakes, and other important factors which are discussed in detail in the latest edition of the ACGIH Handbook on Indus-
trial Ventilation (ACGIH, 1998). Biosafety cabinets should be tested annually by a certified technician.

**Gloveboxes/Aerosol Chambers**

Gloveboxes are often used for toxin operations, especially when protocols require the manipulation of dry powdered toxins (such as weighing operations). Gloveboxes should be hard ducted to the laboratory exhaust ventilation ductwork and should include a differential pressure gauge. Gloveboxes should operate at a minimum of 0.25-inch negative pressure (water gauge) (ACGIH, 1998). For some operations, such as weighing dry powdered toxins, turning off the exhaust ventilation (with installed nonsparking switches) is desirable in order to minimize air currents inside the glovebox.

Gloveboxes in toxin operations should be HEPA filtered, either at the exhaust air plenum or downstream at the exhaust blower. Note that for downstream HEPA filter applications, there is a risk of contaminating ductwork with toxin particulates. For these cases, ductwork should be labeled as potentially contaminated with toxins. A plan should be in place for identifying contaminated ductwork so that future demolition or repair projects are conducted safely.

During temporary openings into a glovebox, such as during glove replacement, an inward airflow of at least 90 linear feet per minute (lfpm) should be maintained. If a glovebox has permanent open areas, it should be evaluated as a fume hood using the recommend parameters for fume hoods below. The interior of gloveboxes should be decontaminated prior to glove replacement.

The selection of glovebox gloves and glovebox gasket material should be based on the chemical(s) used and the permeability of the gloves/gaskets to these chemicals. In some cases, manufacturers use silicone-based gaskets in gloveboxes which may not be suitable if organic chemicals such as solvents are used in the operation. The authors recommend a thorough review of the chemicals, toxins, solvents, and radioactive materials used in a glovebox. Consult the glovebox manufacturer for the selection of gasket material and gloves appropriate to each operation.

Many factors should be considered carefully when designing a glovebox or chamber for aerosol toxin operations. Since gloveboxes or chambers can vary greatly from a few cubic feet for small animal testing to several hundred cubic feet for other applications, one should balance safety/health requirements with ergonomic considerations, operational requirements for the research to be done, and cost.

Ergonomic comfort should be considered when purchasing, modifying, or designing gloveboxes or aerosol chambers. Standard glovebox designs leave much to be desired from the viewpoint of user comfort and range of motion. Consider purchasing or designing chambers that workers can use in a standing or sitting position. For use by a worker in a sitting position, remember to have a cut away or downward bevel in the cabinet floor to allow the worker's legs to slide under the cabinet. An alternative to the standard round gloves are the oblong glove ports that give greater lateral range of motion to the worker's arms, reducing the number of times he or she must reposition to perform a task across the entire glovebox/chamber surface.

A proven and successful approach in facilitating worker access to the glovebox/chamber involves the use of commercially available lightweight half-body suits. The suits are constructed of double-layered material impervious to chemicals including decontaminating solutions and solvents. The suits attach to the bottom interior surface of the chamber using a flange seal, which prevents leakage of contaminants to the outside environment. To use the suit, the worker merely ducks below the chamber floor (beveled entry to reduce stooping) and stands up into the suit. The suit is ventilated by passing HEPA-filtered and conditioned room air through the suit, keeping the operator comfortable in a normal room temperature environment and safe in a positive pressure half-body suit. The suit provides the user with unrestricted access to the glovebox/chamber surface and unimpeded freedom of movement, thus increasing operational efficiency and decreasing fatigue.

Chambers should have dual HEPA filters in series with a differential pressure gauge located before the exhaust filter bank and between HEPA filter banks. Periodic monitoring of the differential pressure gauges will alert operators to particulate "loading" of HEPA filters and/or leakage of the filters due to gasket failure, puncture holes, etc.

Chamber design should also consider the use of attached autoclaves and transfer ports. If large items are to be brought into the chamber (i.e., aerosol fixtures,
mixing tubes, exposure apparatus, plexiglass/Lexan™ containment boxes), a pass through autoclave may be useful as an entry portal. Similarly, with bulk items, it may be more feasible to perform a cursory wipe-down with the decon of choice prior to autoclaving to inactivate the toxins (assuming autoclaving is recommended). Some materials may be very bulky and awkward to effectively decontaminate by hand. An pass-through autoclave attached to the glovebox can enhance both safety and operational ease. The downside is that it increases the footprint size and cost of the chamber and, unless properly vented, can cause heat and condensation to build up in the chamber.

On the other hand, transfer ports are good for passing small-to medium-sized instruments and items in and out of the chamber without risking contamination of the outside environment. If the operator contaminates essential instruments, they can be safely reintroduced into the chamber via the double-flanged ports. Transfer containers holding contaminated material (or whose interior surface is exposed to contaminated air) can be safely opened inside a biosafety cabinet. The interior and exterior of the container and its contents can then be wiped down with decon and used again.

The authors are less enthusiastic about the use of dunk tanks for transferring materials. Problems include:

1. The need to monitor and frequently replace the decon solution
2. The need to carefully consider displacement dynamics with respect to the size of the items to be passed
3. Understanding that merely being submerged in decon for a minute or two is not always sufficient for effective decontamination
4. The difficulty in passing items through the tank
5. Very importantly, the fact that if the tank is not airtight, the pressure differentials between the chamber and the room will tend to push or pull the decon out of the dunk tank.

However, with prudent design and use they can fulfill the operator's needs for safely transferring material between the chamber and the environment.

The authors strongly advocate the "box-in-a-box" concept for conducting intentional aerosol generation tests inside the chamber. A plexiglass/Lexan™ box should be used inside the chamber as the primary containment for generated aerosols. This significantly reduces the contaminated area and makes the decontamination process much simpler. The added safety margin offers a distinct advantage when dealing with large quantities of toxic material and a delivery system, which could easily pose a lethal threat to workers should there be a catastrophic chamber or component failure, coupled with PFC&E failure.

Engineering controls for air pressure differential within the chamber are an important consideration. Depending on anticipated use, it may be desirable to have computerized connectivity between the building HVAC control system and the chamber HVAC elements. While this requires specialized software, it ensures that as building air pressures and differentials shift due to environmental and facility function change, those changes do not interfere with the chamber's function. Additionally, the user has the flexibility to more reliably maintain temperature and humidity inside the chamber and to rapidly use large volumes of air to purge the chamber through the HEPA filters or accommodate tests requiring additional supply air (makeup air) or higher pressure differentials.

 Chambers should use an internal decontamination spray system, which can be operated from outside the chamber. This can best be achieved by use of a revolving high-velocity jet nozzle, which can be initiated remotely. The choice of decon material and concentration should be part of the risk assessment process described earlier.

**Fume Hoods**

Toxic chemical fume hoods are designed to safely ventilate volatile chemical fumes and vapors. Since biological toxins are particulates and do not produce fumes/vapors, fume hoods may not be the best choice for toxin work. In some toxin work scenarios, however, toxic chemical fume hoods may offer adequate protection provided the following criteria are carefully maintained:

- Fume hoods should operate at an average face velocity of 100 linear feet per minute (lfpm) +/- 10%. Higher face velocities may increase air turbulence, thereby reducing the capture efficiency of the fume hood.
- Fume hoods should be used only for toxins in solution. Dry powdered toxins should not be used in a
fume hood since they can be aerosolized easily by the high volume of exhaust air. If there are no other options but to use a fume hood for powdered toxins, a NIOSH-approved, full-face respirator with HEPA canister filter should be worn during the operation.

- Fume hoods used for toxin work should be connected to a HEPA filtration system (preferably dual HEPA in the case of dry powdered toxin work). Almost all fume hoods used in industry, government, and academia for routine chemical operations are vented to the facility exterior via ductwork, but not all of these fume hoods are HEPA-filtered. (Note that in the case of fume hoods, the HEPA filter (if it exists) is located downstream of the fume hood/ductwork, usually on the facility roof or on the ground outside the facility. Due to this configuration, there is a risk of contaminating ductwork with toxin particulates when using fume hoods; therefore, ductwork should be labeled as potentially contaminated with toxins to ensure that future demolition or repair work does not present an exposure hazard to workers.)

- Work with fume hoods should include the 15-cm line concept in the procedures. The 15-cm line is located 15 cm (approximately 6 inches) from the face of the fume hood, usually marked by tape or paint. Work with toxins should be behind the 15-cm line and toward the rear of the fume hood. The area in front of the 15-cm line (from hood face to 15-cm line) is a zone of air turbulence and toxin particulates can easily be ejected from the fume hood into the laboratory by this turbulence. Toxin particulates released behind the 15 cm line are not subject to this turbulence and cannot easily leave the fume hood's air capture.

Laminar flow "clean benches" are designed to protect the product and not the worker; therefore, they are not recommended for work with biological toxins (Richmond & McKinney, 1995).

For all types of engineering controls, periodic examination or testing of joints, seals, and materials that may weaken after repeated use or decontamination is important. This is especially important for chambers used for aerosol studies, glovebox gloves and gaskets, centrifuge rotors, sealed centrifuge cups/carriers, and any equipment that becomes pressurized.

Information on leak test standards for HEPA filters has been published (Institute of Environmental Sciences, 1993; Richmond & McKinney, 1995). Since it may be impractical to decontaminate HEPA filters contaminated with biological toxins, operators should utilize "bag-in"/"bag-out" filter banks to change HEPA filters. Contaminated HEPA filters should be disposed of in accordance with state/local regulations.

**Protective Clothing and Equipment**

Whether working with lyophilized toxins, powdered toxins, or toxins in solution, the risk of ingestion can be mitigated by wearing gloves during work, removing the gloves at the end of the procedure, and washing hands upon exiting the work area. Wearing gloves during work with toxins prevents exposure to toxins, which are absorbed by the skin (such as mycotoxins, Pfiesteria toxins) and protects abraded skin from contact. Similarly, wire mesh, fine metal weave, or Kevlar gloves can be useful for operations involving animals because they reduce both the risks of being bitten and of accidentally inoculating yourself or your assistant.

The use of protective goggles, face shields, safety glasses, and other forms of eye protection is traditionally stressed in chemical laboratories. Such eye protection protects not only against chemicals, infectious agents, and toxins (splashed, sprayed, aerosolized, and dry forms), but also against splashes of hot material, breaking glass, steam leaks, and other industrial hazards which could result in permanent damage or blindness. Close-fitting vented goggles and face shields are recommended for toxin work and generally provide more protection than other forms of ocular PPE, with the exception of full-face respirators.

Whenever a probability of toxin aerosol generation with release associated with engineering failure (work around pressurized vessels such as fermentors, ultracentrifuges, lyophilizers, pharmaceutical bottling operations, etc.) respiratory protection is necessary. Respirators for these scenarios can range from a HEPA-filtered half-face to full-face respirators, to positive pressure respirators with or without protective suits based on the risk of exposure. Respirator selection should be part of the risk assessment and should draw on the advice of safety and health professionals. Respirators should only
be issued to personnel who are medically cleared, have been fit tested, and have been trained in their proper use.

**Recommendations for Personnel Working with Toxins**

Training for toxin workers should include:
- Physical and biological characteristics of the toxins of interest
- Use of PPC&E (including respirator training, proper glove technique, etc.)
- Demonstration of worker proficiency for the planned procedures
- Decontamination procedures
- Signs/symptoms of exposure
- Emergency procedures
- First aid/self-aid
- Prophylaxis/immunization
- Waste disposal

All personnel working with toxins or requiring access to a toxin laboratory should be familiar with the signs and symptoms of toxin exposure. This training should also be provided to first responders, fire department personnel, maintenance/housekeeping personnel, security personnel, and others who must access toxin laboratories, as well as training to local hospitals to provide emergency support.

Personnel working with toxins should participate in periodic training drills where a simulated toxin is spilled or aerosolized in the laboratory. These drills can prove extremely valuable in preparing workers for worst-case scenarios and should include finding exits during power failures, using respirators, using spill kits, and rendering first aid or self-aid. The drills should include safety/health personnel and first responders such as the fire department, medical technicians, and security guards.

Personnel working with toxins should be evaluated for their reliability and qualifications. This evaluation is highly variable from facility to facility and is dependent on factors such as security concerns, potential for terrorist threats, quantity of toxins available to workers, and public relations concerns. A toxin worker reliability/qualification program may include some or all of the following criteria:
- Education and experience
- Formal training in toxin safety
- Immunization for toxins if available (e.g., Botulinum toxins)
- Background police record check
- Random drug/alcohol screen
- Peer review
- Supervisor’s statement of worker reliability

A worker may become temporarily disqualified from toxin operations for certain factors, such as illness, severe stress, or a felony conviction. The decision on when to disqualify or requalify a toxin worker should be made by managers, safety/health personnel, the facility medical authority, and legal professionals.

Entry to toxin labs should be restricted to trained, authorized employees only. The door should be closed and locked for restricted access as necessary. However, ensure that first responders can gain access to locked labs in the event of an emergency. Optimally, medical practitioners familiar with toxin exposure signs, symptoms, and treatment should be available at or near the facility during toxin operations.

Visitors may enter toxin laboratories from time to time, but should always be escorted by authorized personnel. They should not be allowed entry during operations with a potential for personnel exposure, and they should never touch potentially contaminated areas, such as the interior of a biosafety cabinet. Under certain circumstances, they should wear a NIOSH-approved respirator with HEPA canisters (for example, when observing a large-scale toxin aerosol operation in a chamber). Visitors should receive respirator training and be properly fit tested.

**Good Work Practices with Toxins**

**Develop an Operating Procedure**

Procedures should be clear, in chronological order, and include use of protective clothing, decontaminating materials, etc. The operating procedure should be well rehearsed with simulates prior to the start of toxin work (see also Annex 1).
- Allow no eating, drinking, application of lip balms, or storage of food stuffs in the work area. Diligent adherence to these procedures have been shown to
effectively prevent accidental ingestion of laboratory materials.

- Post a sign that states “Caution: Toxin Use Area” or “Caution: Toxin Storage” at entrances to toxin work areas. Only personnel necessary to the operation should be permitted in toxin labs/areas during operations.

- Design toxin protocols should use less than a human lethal dose of toxin whenever possible. As stated previously, the authors recommend biosafety cabinets and gloveboxes for toxin operations; however, one should note that some very dilute/low-level toxin manipulations may present negligible risk to operators and may be suitable for bench top work outside of engineering controls. The risk assessment process will help safety professionals and researchers make sound decisions on the need for the best type of engineering control for each biological toxin protocol.

- Apply a cotton ball or gauze pad (wetted with decontaminating solution) to the junction of the needle and the stopper to prevent any pressurized, aerosolized toxic material from escaping when removing a syringe from a stoppered bottle.

- Use spill trays in fume hoods, gloveboxes, or bench tops for work with toxins in solution. (Note: For biosafety cabinets the work surface acts as a spill tray.) Whenever possible, keep toxin containers in a rack to avoid spills. Even flat-bottomed containers can spill easily and should be secured. Foam blocks cut to fit various container shapes provide an effective means to stabilize containers.

- Take special care when working with powdered or lyophilized materials, as static and ventilation airflow can cause materials very easily and rapidly to form a particulate cloud. Since powdered toxins are highly concentrated, they are likely to be capable of delivering a lethal dose. Try to reduce static by wetting and wiping down gloves and surfaces before starting work. It is best to conduct work in a sealed glovebox or similar engineering control to prevent exposure and dissemination into the room due to ventilation airflow.

- Have a clear and organized work area when injecting animals or otherwise manipulating sharps. Since animals may move or jump just prior to and during injection, it is best to use a restraining device, restrain the animal yourself, or have an inoculation site far from your assistant’s hands/body. Practice the procedure in “dry runs” until the technique is familiar and comfortable.

- Do not store or consume foods or beverages in toxin work areas. Do not smoke, chew gum, or apply cosmetics.

- Store toxins in double containment configuration. The outer container should be leak-proof and unbreakable. Toxin storage areas should maintain an inventory of toxins and quantities of each toxin.

- Minimize the use of sharps. If there is no alternative to using sharps in a protocol, follow the sharps guidelines in *Biosafety in Microbiological and Biomedical Laboratories* (Richmond & McKinney, 1993).

- Use caution when working with disposable pipette tips. Pipette tips can be easily ejected from a biosafety cabinet or fume hood during toxin operations. Tips should be ejected into a container of decon solution in the fume hood/biosafety cabinet.

- Follow the “two-person” rule; when appropriate. This requires that two authorized persons be present during toxin operations and is especially critical when the protocol requires work with greater than a human lethal dose of toxin or for any operation where toxin aerosolization is probable or intentional.

- Prevent cross drafts and air turbulence, which may be caused by air circulating fans, window air conditioning units, or open windows and may defeat the capture efficiency of fume hoods or biosafety cabinets. To prevent cross drafts and air turbulence, air circulating fans and window air conditioning units should not be used during toxin operations. Windows should be closed during toxin operations.

- Keep fit-tested, NIOSH-approved (or equivalent) full-face respirator with HEPA cartridge filter on hand for each operator for emergency egress, first aid, and emergency spill response as determined by the risk assessment.

- Wear a lab coat during toxin operations. Lab coats should be kept in the laboratory until turned in for laundering.

- Wear goggles and face shield during toxin work.

- Perform a preoperational check prior to each day’s operation. The pre-op check should ask (at a mini-
mum) Is adequate protective clothing available? Is adequate decontaminating material on hand? Is the lab phone operational? Is the fume hood/biosafety cabinet/glovebox operational? Are spill kits available? Is the lab entrance posted? Are exhaust system and alarms functional? See Figure 2 for a suggested preoperational checklist.

- Use the 15-cm line concept for all work with tox-

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**Figure 2**
Suggested Preoperational Checklist for Toxin Work

**Facility:**
- Ensure lab is under negative pressure.
- Lab entrance warning signs posted
- Emergency shower/eyewash operational
- Ventilation system alarm operational
- Ensure telephone is operational (for emergency notification).

**Engineering Controls:**
- Fume hood operational within certification and at 100 lpm
- 15 cm line present, all toxin containers behind 15 cm line
- Biosafety cabinet operational within certification
- Glovebox operational and at 0.25" negative pressure
- Class III biosafety cabinet operating at 0.50" negative pressure
- Excess materials/equipment removed from hood/biosafety cabinet

**Protective Clothing/Equipment:**
- Gloves available and worn (2 sets minimum)
- Chemical splash goggles and face shield available and worn
- Lab coats available and worn
- NIOSH-approved respirator with HEPA filter available for each toxin worker
- Respirator fit tested and workers trained in use

**Personnel Requirements:**
- Do personnel have adequate training/experience for the operation?
- Personnel familiar with signs/symptoms of exposure
- Personnel immunized (if applicable)
- Personnel medically cleared for work
- Personnel trained in first aid/buddy aid

**Work Practices:**
- Medical coverage available during operation
- Risk assessment and operating procedure approved
- Appropriate decon for toxin(s) available
- Toxin quantity is smallest amount needed for the operation.
- Two-person rule followed
- Gloves leak tested
- Waste and decon containers available
- Spill kit available

Date: __________ Primary operator: __________________ Secondary Operator: __________________
ins in fume hoods or biosafety cabinets. See discussion on 15-cm line under Engineering Controls, Fume Hoods.

- Do not perform manipulations of dry powdered toxins in a fume hood or Class I or Class II biosafety cabinet. Use a glovebox, Class III biosafety cabinet, or preferably introduce a buffer to the dry powder to bring the toxin into solution. Fume hoods and Class I and Class II biosafety cabinets can aerosolize and disperse dry powdered toxins.
- Pressure test gloves prior to use to check for leaks. This can be accomplished by blowing into the glove to trap air and observing for leaks. After working with toxins, remove gloves in a manner as to prevent contamination of hands.
- Wash hands after toxin work is completed and prior to eating, drinking, smoking, or applying cosmetics.

Decontamination Recommendations (Eitzen, et al., 1998; Whalley, 1990)

The preferred decontamination method for toxins varies widely with the particular toxin and the area requiring decon (such as personnel, areas, or equipment). Researchers and safety professionals should review the available literature for each toxin of interest and determine the best decon methodology for the operation as part of the risk assessment process. Also ensure that decontaminating solutions are the proper concentration. Stock 5% sodium hypochlorite (household bleach) is widely used as a decontaminating material, but it loses its active chlorine content with time and should be tested and/or replenished frequently.

Personnel Decontamination

In most cases, the first step in personnel decontamination is clothing removal, followed by a wash of the contaminated area (not eyes, however) with 0.5% sodium hypochlorite solution or soap and water. Eye splashes should be immediately treated with copious amounts of water from an eyewash station (in the case of mycotoxins, preferred treatment is with copious amounts of saline solution). For large quantities of toxin (liquid) on clothing or skin, immediately utilize an emergency deluge shower, then follow up with 0.5% sodium hypochlorite or soap and water.

Equipment/Work Surfaces

For most toxins, 0.5% sodium hypochlorite is an effective decontaminant for equipment and work stations. However, as mentioned above, the preferred decon is highly variable from toxin to toxin. For example, SEB is resistant to active chlorine decontaminating trials but is effectively decontaminated with formaldehyde. Mycotoxins are best deconned with a mixture of 1.0% sodium hypochlorite and 0.1M NaOH with 1-hour contact time. The bottom line is: Review the available literature to find the most effective decontaminant for the toxin of interest.

Glassware

Soak all-toxin contaminated glassware in a mixture of 2.5% sodium hypochlorite and 0.25 N NaOH for 8 hours. As an alternative, soak in 5% sodium hypochlorite for 8 hours.

Solid Waste

Waste, such as gloves, gauze pads, etc. should be soaked in a mixture of 2.5% sodium hypochlorite and 0.25 N NaOH for 16 hours or more. As an alternative, soak in 5% sodium hypochlorite for 16 hours or more. Using tongs or forceps, separate waste from liquid and air-dry in a fume hood or biosafety cabinet. Double bag the dried waste, label as hazardous waste, and dispose of in accordance with state/local requirements.

Liquids

Decon toxin solutions 1:1 with a mixture of 2.5% sodium hypochlorite and 0.25 N NaOH, mix well, and hold for 8 hours. Label as hazardous waste and dispose of in accordance with state/local requirements.

Emergency Response Recommendations

The risk assessment should address emergency procedures specific to the operations, the type of toxin(s)
used, and the configuration of the facility. The following criteria may be a helpful starting point for developing an emergency response plan:

- Medical support should be readily available prior to the start of toxin operations.
- Phone numbers of emergency response personnel, fire department, clinic, etc. should be posted at the worksite. Ensure phone is operational prior to start of operations.
- First aid: Exposed personnel should be immediately moved to an uncontaminated area. When providing assistance, personnel should wear the appropriate level of protective clothing and equipment to avoid becoming exposed to toxins. The selection of PPCS&E for emergency response depends on the type of toxin, quantity of toxin used, powder form versus liquid form, and other factors. At a minimum, use gloves and protective eyewear, such as goggles. A full-face, NIOSH-approved respirator with HEPA filter canister may be indicated for some emergency response /first aid scenarios.
- Skin contamination: See Decontamination Recommendations above.
- Eye/mucous membrane contamination: See Decontamination Recommendations above.
- Toxin spills: Rinse gloves with decon solution and remove. Put on new gloves. Cover spill with absorbent pad or pillow premoistened with appropriate decontamination solution. Avoid flooding the area with decontaminant since this will spread the contamination. Keep others away from the spill area until the decon procedure is complete. Wait at least an hour before entering the lab without a respirator (allows sufficient air changes to purge the room).
- Fume hood/biosafety cabinet failure: Close or cover all toxin containers. Shut down operations, close hood sash, and leave room. When the fume hood/biosafety cabinet is back on line, wait at least an hour before entering lab without a respirator (allows sufficient air changes to purge the room).
- Building power failure: Close or cover all toxin containers. Shut down operations, close the hood/biosafety cabinet sash, and leave room. When power is back on line, wait at least an hour before entering lab without a respirator (allows sufficient air changes to purge the room).

Security Considerations for Toxin Laboratories

Security requirements vary widely based on quantity of toxin in storage, types of toxins, location of the facility, perceived potential for terrorist threat, and public perception. A security program for toxins should be documented and should consider some or all of the following criteria:

- Toxin storage areas (such as freezers) should be locked at all times. The facility should institute a key control plan.
- Facilities with a high public relations risk factor or a concern of industrial espionage or other types of threats can employ a motion detection system with alarms for off-duty surveillance hours.
- A facility security force, punch locks, card readers/ palm print readers, or similar devices may be used in scenarios where a higher level of security is desired. Many modern security systems can incorporate training data, medical data, and other worker information into the security system to restrict access to toxin work areas.

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References


Safety and Health Considerations for Conducting Work with Biological Toxins


Annex I
Suggested Checklist for Safety Surveys of Toxin Work Areas

Date: ___________________________    Building/Room: ___________________________

Surveyor: _________________________    Lab Supervisor: _____________________________

[ ] Yes [ ] No  Are all operations with toxins being conducted inside certified biological safety cabinets, fume hoods, or gloveboxes?
[ ] Yes [ ] No  Have biosafety cabinets, gloveboxes, and chemical fume hoods been certified in the last 12 months?
[ ] Yes [ ] No  Are all containers of toxins appropriately labeled? Is a list of toxins posted in the lab?
[ ] Yes [ ] No  Are freezers, refrigerators, and storage units labeled with biohazard warning sign?
[ ] Yes [ ] No  Are the eyewash and emergency shower unobstructed, tested, and ready for use?
[ ] Yes [ ] No  Are all reagents and solutions properly labeled?
[ ] Yes [ ] No  Is a spill kit within a reasonable distance from the work areas?
[ ] Yes [ ] No  Is appropriate protective clothing available for the hazards present?
[ ] Yes [ ] No  Are MSDSs or Toxin Summary Sheets located where they are available to the laboratory workers?
[ ] Yes [ ] No  Are storage and shipping containers adequate and properly labeled?
[ ] Yes [ ] No  Are personnel immunized (if applicable)?
[ ] Yes [ ] No  Have all personnel been adequately trained in biosafety?
[ ] Yes [ ] No  Are laboratory doors kept closed when experiments are in progress?
[ ] Yes [ ] No  Are all operations conducted over plastic-backed absorbent paper or spill trays?
[ ] Yes [ ] No  Are all floor drains sealed and filled with water or suitable disinfectant?
[ ] Yes [ ] No  Is an operating procedure posted and signed by personnel working with toxins in the room?
[ ] Yes [ ] No  Is the appropriate decon available (bleach, 5% NaOH, etc.)?
[ ] Yes [ ] No  Are all entrances to the laboratory posted with the appropriate special provisions for entry, the universal biohazard symbol, and the name/number of lab supervisor?
[ ] Yes [ ] No  Are phones operational and are emergency phone numbers posted in the lab?
[ ] Yes [ ] No  Is entry limited and restricted?
[ ] Yes [ ] No  Are gloves worn when handling toxins or animals?
[ ] Yes [ ] No  Are all vacuum lines protected with HEPA filters and liquid disinfectant: traps?
[ ] Yes [ ] No  Are windows and penetrations through the walls and ceilings sealed?
[ ] Yes [ ] No  Is lab under negative pressure relative to all entrances?
[ ] Yes [ ] No  Is the autoclave properly maintained and certified?

General Housekeeping:
[ ] Yes [ ] No  Is the room free of clutter? Are all aisles from the work areas to available exits clear of obstructions?
[ ] Yes [ ] No  Are all safety equipment items unobstructed and ready for use?

Fire Safety:
[ ] Yes [ ] No  Is the fire extinguisher hung in its proper place, ready for use, and unobstructed?
[ ] Yes [ ] No  Are excess flammables located outside NFPA-approved cabinetry?
[ ] Yes [ ] No  Are all Class IA/IB flammables that are in breakable containers in pint or smaller containers?
[ ] Yes [ ] No  Are chemicals stored with compatible materials?

General Laboratory Safety:
[ ] Yes [ ] No  Are sharps discarded and destroyed in a safe manner?
[ ] Yes [ ] No  Are work surfaces decontaminated daily and after a spill?
[ ] Yes [ ] No  Is the appropriate attire worn by everyone in the room?
[ ] Yes [ ] No  Is there evidence that personnel eat, drink, smoke, or store food, drinks, or tobacco in the room?
[ ] Yes [ ] No  Are all gas cylinders secured and are all cylinders not in use capped?
[ ] Yes [ ] No  Was any unsafe procedure observed (such as mouth pipetting, work without gloves, etc.)?

Notes: