DRAFT Guidelines for Laboratories Working with Microbial Toxins

1. Introduction

Microbial toxins consist of any toxic substance produced by a microorganism. They can cause acute toxic disease as well as long-term effects(1, 2).

Although there is an abundance of information and many regulations concerning both biosafety and chemical safety, those addressing toxins are limited. One reason for this paucity is that toxins do not fit neatly into either category but share some aspects of both(2).

Toxins are nonreplicating and are not communicable between individuals, unlike pathogenic organisms. They are, however, capable of eliciting pathologic effects associated with infectious diseases, and are one of the key virulence factors(1,2, 9). Most toxins are highly toxic in minute quantities and represent an airborne particulate hazard to workers (2). There are no short-term exposure limits, ceiling limits, or time weighted averages (TWA) for toxins, and toxicological data are often limited. There are also no environmental monitors for toxins currently available(2). These are important differences distinguishing toxins from chemical hazards. Toxins are generally not volatile or dermally active (some mycotoxins are exceptions), therefore they are most likely to represent a hazard through aerosolization, ingestion, or percutaneous injection(1,2). Using features of both chemical and biological safety programs together with a zero exposure approach presents an effective way to mitigate the risk involved in working with microbial toxins. Special care must be taken when working with multiple toxins, as toxin-toxin interactions leading to synergistic, zero, and antagonistic effects and subthreshold combination effects could occur(3-5). If other hazards, such as infectious agents, are also being used in the same laboaratory, consideration must be given to physical and operational controls for both hazards.

An overview of many aspects of microbial toxins, including information on the modes of action of the organism/toxin, disease, signs and symptoms in humans, diagnosis, and treatment can be found in the references cited below:

2. Scope

These draft Guidelines for Laboratories Working with Microbial Toxins are intended to specifically address the operational and physical requirements for laboratories working with exotoxins (including enterotoxins) produced by microorganisms, primarily bacteria, and generally address work with mycotoxins. Laboratories unable to meet the requirements outlined in the Guidelines should contact our Office by phone, fax, mail or email, for further guidance.

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These guidelines are not intended for work with chemical toxins or chemicals. For guidance on work with these agents, the reader may refer to Provincial and Federal Workplace Hazardous Materials Information System documentation as well as Provincial Occupational Health and Safety Legislation guidance documents. The U.S. Center for Disease Control's draft Chemical Safety Levels (CSL's): A proposal for chemical safety practices in microbiological and biomedical laboratories is also available at: http://www.cdc.gov/od/ohs/CSL%20article.htm.

3. Risk Assessment

As each laboratory program employing toxins will vary slightly 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 consider the following(2):

- Probability of aerosol generation;
- Amount of toxin being worked with;
- Availability of prophylaxis and/or treatment;
- Training, experience of personnel and accident records;
- Intoxication/lethality dose data;
- Health effects data for exposure;
- Engineering controls;
- Safety equipment availability and efficacy;
- Personal protective equipment (clothing and equipment) availability and efficacy;
- Identification of specific hazards within the protocol and mitigation of these hazards prior to work commencement.

4. General Safety Practices

Since the transmission and routes of entry of toxins resemble those of microorganisms, Containment Level 2 facilities using Containment Level 3 operational practices are generally appropriate. The operational practices and physical requirements listed in Chapters 3 and 4 of the Laboratory Biosafety Guidelines, 3rd Edition, 2004 (http://www.phac-aspc.gc.ca/ols-bsl/lbg-ldmbl/index.html) should be reviewed and applied where appropriate.

In addition the following practices should be followed when working with microbial toxins.

1. A toxin safety protocol is to be developed that covers each toxin to be worked with. The plan is to include:
    a. standard operating procedures covering all aspects of toxin work, including basic handling and experimental protocols; procurement,

distribution, and storage; and decontamination, detoxification, and disposal;
   b. a list of all potential hazards associated with normal use, and those that could result from a spill or an accident;
   c. policy and practice in place to minimize risk, including facility requirements, personal protective clothing and equipment, spill and accidental exposure management;
   d. a medical program that would cover a medical surveillance plan, first aid, and any special conditions or exclusions for working with the toxin (e.g. immunization);
   e. documented training specific to the toxin for all individuals who will be working with it.

2. Inventory control is to be in place and regularly updated. Toxins are to be stored in locked facilities (cabinets, lockers, freezers) that are restricted to authorized personnel.
3. Toxins are to be transported in spill and leak proof secondary containers.
4. Preparation and manipulation of toxin stock solutions and primary containers of dry toxins as well as other high-risk procedures is to be conducted in a biological safety cabinet (BSC), or glove box approved by the Biological Safety Officer. Please refer to Chapter 9 of the Laboratory Biosafety Guidelines, 3rd edition for a more detailed discussion of biological safety cabinets. Exhaust must be HEPA filtered; charcoal filters may also be required if volatile substances are used. Inspection of proper airflow and work within the operationally effective zone is to be adhered to.
5. It is not recommended that chemical fume hoods be used for dry powdered toxins as they can be aerosolized easily due to the high volume of exhaust air. If no other option is available, it is recommended that face protection and N-95 respiratory protection be employed. Please note that the chemical fume hood exhaust must be HEPA filtered.
6. Every effort is to be made to work with less than one human lethal dose of toxin.
7. Two knowledgeable individuals should be present in the laboratory during toxin operations whenever high-risk procedures are performed. Such procedures include, but are not limited to, working with more than one human lethal dose; intentional creation of aerosols; working with powdered/lyophilized toxins; creating primary containers; and work involving a fast acting toxin. Both individuals are to be familiar with all the procedures being performed, the signs and symptoms of possible exposure, and emergency response and first aid; both workers are to remain within eye contact to be able to assist in the result of an accident or incident[1,2,6-8].
8. Signs are to be posted at laboratory entrances indicating that toxins are in use, restricting access to authorized personnel, and listing any special entry requirements.
9. Before removal from containment, primary containers are to be decontaminated and placed within clean secondary containers.

10. Personal protective clothing is to be decontaminated before disposal or laundering; if this is not possible it should be disposed of as hazardous waste.
11. The interior of the containment device (hood, cabinet, box) is to be decontaminated at regular intervals; signs indicating that toxins are in use are to be placed on cabinets that have not been decontaminated.
12. Laboratory personnel are to use long-sleeved, disposable body covering and appropriate gloves.
13. Gloves are to be inspected and pressure tested for leaks before use; care is to be taken when selecting glove material; when the work involves powdered toxins, glove material (not latex) should be selected to minimize static electricity; gloves and surfaces may be dampened to reduce static electricity; glove material is also to be impervious to the toxin being worked with and any diluents (1,2,6-8).
14. Deluge showers and eyewash stations are to be available in laboratories where toxin work takes place; when toxins readily absorbed through the eyes are used, eyewash stations should be in place near every work area.

5. Decontamination

1. Decontamination procedures (e.g. autoclaving, chemical disinfectants) should be assessed for each toxin by consulting current literature, as the optimal procedures for each toxin vary widely. Decontamination solutions should be routinely tested to ensure that they are at the proper concentrations. Many commonly used agents can rapidly lose their activity. When working with infectious organisms in conjunction with their toxins, care must be taken to ensure that both the infectious agent and the toxin are neutralized.

2. For most protein toxins, a sodium hypochlorite, or sodium hypochlorite and sodium hydroxide mixture provides effective decontamination. Surfaces may be decontaminated with a 0.5% solution of sodium hypochlorite. Solid and liquid waste may be decontaminated with a solution of 2.5% sodium hypochlorite and 0.25 N sodium hydroxide. They should be soaked or mixed in a 1:1 ratio and allowed to stand for 5 or 8 hours respectively(2). Again it must be noted that the preferred method is highly variable and should be ascertained for the specific toxin in question.

6. References

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7. *Biological defense safety program, technical safety requirements*. Title 32. Code of Federal Regulations Part 627, 892.
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