CHAPTER IX: BIOLOGICAL TOXINS

BASIC CHARACTERISTICS

Biological toxins are natural, poisonous substances produced as by-products of microorganisms (exotoxins, endotoxins, and mycotoxins, such as T-2 and aflatoxins), plants (plant toxins such as ricin and abrin), and animals (zootoxins such as marine toxins and snake venom). Unlike pathogenic microorganisms, including those that produce toxins, the toxins themselves are not contagious and do not replicate. In this regard, toxins behave more like chemicals than infectious agents. However, unlike many chemical agents, biological toxins are not volatile and are odorless and tasteless. The stability of toxins varies greatly, depending on the toxin structure (low molecular weight toxins are quite stable).

Most biological toxins, with the exception of T-2 Mycotoxin, are NOT dermally active; i.e., intact skin is an excellent barrier against most toxins. That said, mucous membranes of the eyes, nose, and mouth serve as portals of entry, as do breaks in the skin. Aerosol transmission, ingestion, and percutaneous transmission are also a concern for most biological toxins.

Bacterial toxins can be exotoxins (including enterotoxins) or endotoxins. Exotoxins are cellular products excreted from certain viable Gram-positive and -negative bacteria, highly toxic (i.e., microgram quantities) and are relatively unstable (destroyed rapidly when heated to $\geq 60^{o}C$). Bacterial endotoxins are lipopolysaccharide complexes derived from the cell membrane of Gram-negative bacteria that are released upon bacterial death. Endotoxins are relatively stable (can withstand heating at $60^{o}C$ for hours without losing activity) and moderately toxic (tens to hundreds of micrograms required for animal fatality).

The modes of action of biological toxins vary, but include damage to cell membranes or cell matrices (e.g., *Staphylococcus aureus* alpha toxin), inhibition of protein synthesis (e.g. Shiga toxin), or via activation of secondary messenger pathways (e.g. *Clostridium botulinum* and *C. difficile* toxins).

LABORATORY REQUIREMENTS AND SAFETY OPERATIONS

Most work with biological toxins can be safely managed in a BSL-2 setting. In some cases (e.g., large scale production, manipulation of large quantities of powder form of toxin) management at BSL-3 may be required, depending on the toxin in question and the quantities used. The most hazardous form of any toxin is the dry, powder form. Manipulations of dry forms of toxins should be performed in a biological safety cabinet or in a fume hood. In some cases a glove box may be recommended for such operations.

Once reconstituted into an aqueous form, BSL-2 management is usually sufficient for work with most biological toxins. Access to the lab should be controlled when toxin is in use. Biohazard warning signs displaying the biosafety level, toxin in use, emergency contact information, and entrance requirements (available upon request from the Office of Biological Safety) should be posted at the lab entrance. If vacuum lines are used, it is advisable to protect the vacuum system with an in-line disposable HEPA filter. Personal protective equipment should include a lab coat, gloves, and mucous membrane protection. You should routinely confirm the operational status of your lab eye-wash station and safety shower. All personnel in the lab should be trained about the specific hazards associated with the toxin in use. At UC, an IBC protocol is required for research utilizing any of the toxins listed in Table 4.

Table 4
TOXINS THAT REQUIRE AN IBC PROTOCOL

Toxin	LD_{50} (µg/kg)*
Abrin	**0.7**
Aerolysin	**7**
Botulinum toxin A	**0.0012**
Botulinum toxin B	**0.0012**
Botulinum toxin C1	**0.0011**
Botulinum toxin C2	**0.0012**
Botulinum toxin D	**0.0004**
Botulinum toxin E	**0.0011**
Botulinum toxin F	**0.0025**
b-bungarotoxin	**14**
***Clostridium difficile* enterotoxin A**	**0.5**
***Clostridium perfringens* lecithinase**	**3**
***Clostridium perfringens* perfringolysin O**	**13-16**
***Clostridium perfringens* delta toxin**	**5**
Clostridium perfringens epsilon toxin	**0.1**
Conotoxin (Only short, paralytic alpha conotoxins with specific sequences are considered Select Agents)	**12-30**
Diacetoxyscirpenol	**1000-10,000**
Diphtheria toxin	**0.1**
Listeriolysin	**3-12**
Modeccin	**1-10**
Pertussis toxin	**15**
Pneumolysin	**1.5**
***Pseudomonas aeruginosa* toxin A**	**3**
Ricin	**2.7**
Saxitoxin	**8**
Shiga toxin	**0.25**
***Shigella dysenteriae* neurotoxin**	**1.3**
***Staphylococcus* enterotoxin B**	**25**
***Staphylococcus* enterotoxin F**	**2-10**
***Staphylococcus* enterotoxins A, C, D, and E**	**20(A); <50(C)**
Streptolysin O	**8**
Streptolysin S	**25**
T-2 toxin	**5,000-10,000**
Taipoxin	**2**
Tetanus toxin	**0.001**
Tetrodotoxin	**8**
Volkensin	**1.4**
***Yersinia pestis* murine toxin**	**10**

*Note that the LD_{50} values are from a number of sources (see below). For specifics on route of application, animal used, and variations on the listed toxins, please go to the references listed below. (Table courtesy, in part, of University of Florida EHSO).

Toxins noted in RED are considered Select Agents if being stored in large enough quantities (see Chapter X below). For more information please consult:
http://www.selectagents.gov/Permissible%20Toxin%20Amounts.html

REFERENCES:
1. Gill, D. Michael; 1982; Bacterial toxins: a table of lethal amounts; Microbiological Reviews; 46: 86-94.
2. Stirpe, F.; Luigi Barbieri; Maria Giulia Battelli, Marco Soria and Douglas A. Lappi; 1992; Ribosome-inactivating proteins from plants: present status and future prospects; Biotechnology; 10: 405-412.
3. Registry of toxic effects of chemical substances (RTECS): comprehensive guide to the RTECS. 1997. Doris V. Sweet, ed., U.S. Dept of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Cincinnati, OH.

SECURITY

It is important that stocks of biological toxins be maintained in locked cabinets, freezers, and/or refrigerators. Since biological toxins are not self-replicating as are microorganisms, it is prudent to maintain an inventory of toxins present in a lab at any given time. This inventory should display the current quantity of a particular toxin on-site, the date and amount removed from storage, the person removing the aliquot from storage, the purpose of use, and the quantity remaining. *Toxin Inventory Forms* are available from the Office of Biological Safety upon request.

DECONTAMINATION METHODS

The majority of biological toxins can be inactivated or decontaminated with household bleach or autoclaving. Tables 5 and 6 describe the inactivation regimens for biological toxins in common use:

Table 5
COMPLETE INACTIVATION OF DIFFERENT TOXINS WITH A 30-MINUTE EXPOSURE TIME TO VARYING CONCENTRATIONS OF SODIUM HYPOCHLORITE (NaOCl) +/- SODIUM HYDROXIDE (NaOH)

Toxin	2.5% NaOCl[a] + 0.25 N NaOH	2.5% NaOCl[a]	1.0% NaOCl[b]	0.1% NaOCl[c]
T-2 Mycotoxin	YES	NO	NO	NO
Brevetoxin	YES	YES	NO	NO
Microcystin	YES	YES	YES	NO
Tetrodotoxin	YES	YES	YES	NO
Saxitoxin	YES	YES	YES	YES
Palytoxin	YES	YES	YES	YES
Ricin	YES	YES	YES	YES
Botulinum	YES	YES	YES	YES

(Wannemacher 1989)
[a]2.5% NaOCl is approximately equal to 50% household bleach (1:2 dilution)
[b]1.0% NaOCl is approximately equal to 20% household bleach (1:5 dilution)
[c]0.1% NaOCl is approximately equal to 2% household bleach (1:50 dilution)

Table 6
COMPLETE INACTIVATION OF TOXINS BY AUTOCLAVING OR 10-MINUTE EXPOSURE TO VARYING TEMPERATURES OF DRY HEAT

Toxin	Autoclaving	Dry Heat°F			
		200	500	1000	1500
T-2 Mycotoxin	NO	NO	NO	NO	YES
Brevetoxin	NO	NO	NO	NO	YES
Microcystin	NO	NO	YES	YES	YES
Tetrodotoxin	NO	NO	YES	YES	YES
Saxitoxin	NO	NO	YES	YES	YES
Palytoxin	NO	NO	YES	YES	YES
Ricin	YES	YES	YES	YES	YES
Botulinum	YES	YES	YES	YES	YES

(Wannemacher 1989)

For exposure events involving skin exposure to minute quantities of toxin, soap and water are effective in removing the toxin burden (toxins are not dermally active, except for T-2 mycotoxin). For significant exposures to biological toxins, contact Occupational Medicine immediately.