GUIDELINES FOR WORKING WITH MICROBIAL TOXINS

SCOPE

This is a guideline document for working with biological toxins in accordance with the Human Pathogen and Toxin Act (HPTA) and Regulations (HPTR). Plant toxins and animal/insect venoms are not included as part of the HPTR.

PURPOSE

The purpose of this document is to provide general guidance on:

- Biological Toxins requiring security clearance
- Safe work practices with biological toxins
- Inactivation and disposal of biological toxins

BIOLOGICAL TOXINS

Biological toxins are poisons, either naturally derived from an animal, plant or microbial source or synthetically derived, that cause death or severe incapacitation at relatively low exposure levels. These biological substances are regulated by the Human Pathogens and Toxins Act (HPTA) and Regulations (HPTR) to protect the health and safety of the public. The HPTA Regulations and the Canadian Biosafety standards address the risks associated with several aspects of pathogens and toxins affecting human health, whether imported or domestically acquired, including the disposal of toxin waste. Toxins are classified as toxic substances (class 6.1) under Canada's current Transportation of Dangerous Goods Regulations.

Toxins do not replicate and they are not infectious. In amounts used most typically in biomedical research laboratories, toxins can be handled safely by trained laboratory personnel and they are a negligible risk to the local community. However, they have very low LD50s and can cause acute health effects including incapacitation, or even death in humans and animals. Microbial toxins pose a small but significant risk to human health and safety, either through accidental or deliberate release. Route of exposure to biological toxins in the laboratory include:

- Accidental inoculation
- Absorption through the skin or mucous membrane
- Ingestion and
- Inhalation of aerosols

SECURITY SENSITIVE BIOLOGICAL AGENTS

Security sensitive biological agents, or SSBAs, are a subset of Risk Group 3 and 4 human pathogens and prescribed toxins that are included in Schedule 1 of the Human Pathogens and Toxins Regulations (HPTR) and also on the Australia Group "List of Human and Animal Pathogens and Toxins for Export Control" (AG List). If they are misused, SSBAs can pose a risk to Canada's national security, which is why individuals who work with them will be assessed through the HPTA Security Clearance process. The Public Health Agency of Canada has prepared a list of toxins that will require an HPTA Security Clearance if an individual is in possession of a toxin in an amount that exceeds the trigger quantity stated in section 10 of the HPTR. Individuals working with toxins in quantities less than the indicated trigger quantity will not require an HPTA Security Clearance. See Appendix 1 for the list of HPTA regulated biological toxin, security-sensitive status, and their t.

SAFE WORK PRACTICES

Local Risk Assessment: The risk associated with microbial toxins will vary depending upon the nature and amount of toxin used, and the procedure being performed. Therefore, 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 for working with microbial toxins should include:

- Probability of aerosol generation
- Amount of toxin being worked with
- Availability of prophylaxis and/or treatment
- Training, the 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

General Safe Work Practices: Based on the risk assessment, the lab should develop an SOP or a work instruction for the lab-specific work with the microbial toxin. However, there are some standard safe work practices and procedures that all the labs working with microbial toxins should follow and should be a part of lab-specific SOP:

- The minimum containment level for handling and storing the majority of regulated biological toxins is Containment Level 2 (CL2).
- 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.

- Good microbiological techniques must be applied at all times.
- Always work with less than 1/10th of one LD50 of the toxin.
- Material Safety Data Sheet (MSDS) / Safety Data Sheet (SDS) should be available to staff all the time.
- Conduct all preparation and dispensing of biological toxins in a certified Biosafety Cabinet (BSC).
- Avoid working with powders whenever possible. If however, this is not possible, reconstitute the entire vial of powdered toxin by injecting the diluent through the septum. Do not remove the cap or attempt to reconstitute only a portion of the vial content.
- Place bench coat or absorbent pad on the BSC work tray before starting and dispose of it when finished in the designated cytotoxic waste container.
- Before removing toxins from the BSC, decontaminate the exterior of the primary container and place it in a clean secondary container.
- All potentially contaminated disposable items (such as gloves used in preparation) must be placed in a hazardous waste bag and autoclaved before disposal.
- Transport and store powders and concentrated stock solutions of biological toxins in a leak and spill-proof secondary container.
- Avoid the use of 'Sharps' such as needles, scalpels, and surgical instruments. Also, use plastic-ware and disposable transfer pipettes instead of glassware and Pasteur pipettes.
- Use centrifuge safety cups or sealed rotors when centrifuging cultures or materials containing toxins and ensure tubes are thick-walled and have a screw-capped top. Remove the entire sealed rotor or centrifuge safety cups into the BSc to open and remove tubes.
- Wear double gloves and before removing hands from the BSC, remove the outer pair and dispose of it in a bag/container within the cabinet.
- Toxin and the contaminated waste removed from the BSC should be treated as Cytotoxic waste and place it in the designated box or Chemogator (Sharps).
- 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.
- Toxins are to be transported in a spill and leak-proof secondary containers.
- 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, 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.
- Gloves are to be inspected and pressure tested for leaks before use. The glove material should be selected carefully. When the work involves powdered toxins, glove material (not

latex) should be selected to minimize static electricity and 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.

- Wash hands upon task completion and glove removal or any time gloves have been compromised.
- Appropriate supplies for the spill cleanup of the specific toxin should be maintained in the clearly marked spill cleanup kit

The above practices are basic safe work practices for working with any microbial toxin. Each lab should develop its own Risk Assessment and Safe Work Procedure for the specific toxin and the procedure being performed.

SPILL CLEAN UP

All spills involving microbial toxin must be reported to the Biosafety Office.

General Spill Cleanup: Liquid spill and powder spill inside the BSC or containment can be cleaned by following the Biological Spill Clean-up SOP. It is important to remember that the inactivation of the microbial toxin is different from the inactivation of a biological agent. Refer to Appendix 3 for the best disinfectant and the contact time for microbial toxin spill cleanup. Alternatively, check the literature for the most appropriate inactivation method. In case of spill inside the BSC, it is important to keep the cabinet running for a longer time 1 hour after spill cleanup.

Powder Spill outside Containment: For the powder spill outside of BSC or containment it may be necessary to call 911 if there is potential for exposure (for instance if individuals present are not wearing respirators and eye protection) or no one is capable of safe spill cleanup.

- Remove all personnel from the laboratory and restrict access.
- Access the extent of the spill and characteristics of the toxin involved in the spill. Determine if the lab has the ability/resources to carry out the spill cleanup.
- **All the personnel involved in spill cleanup should wear respirator** along with other required PPE. Thus, the lab members who could be involved in spill cleanup should get fit tested for the respirator in advance and the respirator should be readily available.
- **If the spill cleanup is beyond the lab's capacity, contact Biosafety Office (604-209-3199 and 911)**; tell them that a highly toxic spill has occurred, and you need assistance to contain it.
- Be prepared to provide the following information:
- Name and phone number of the knowledgeable person that can be contacted
    - Name of toxin, concentration and amount spilled
    - Number of injured, if any
    - Location of spill

INACTIVATION AND DISPOSAL

All the microbial waste must be inactivated before disposal.

Inactivation

Inactivation of microbial toxin means to render the toxin nonfunctional so that it is no longer capable of exerting its toxic effect. This is different from the inactivation of biological agents, which renders the agent non-viable, or no longer capable of growing, replicating, infecting, or causing disease. Inactivation methods used for microbial toxins must be specific for the toxin. Microbial toxins can be inactivated by using heat (for example autoclave, incineration) or chemicals (for example sodium hypochlorite, sodium hydroxide).

Biological toxins have a wide variety of physical properties, thereby making it impossible to provide standard inactivation procedure for all circumstances. The responsibility of the laboratory or facility that handles and/or stores toxins to determine the most effective inactivation method. The waste generator should review the toxin Safety Data Sheet (SDS) to determine the appropriate means of inactivation and follow the disposal method listed in the approved Biosafety protocol. Thus, the first step in inactivation and disposal is to determine the appropriate inactivation method for the toxin: moist heat (autoclaving), chemical inactivation, or incineration. Refer to Appendix 2 and Appendix 3 for details on heat and chemical decontamination of common microbial toxins.

Autoclaving: Inside a fume hood or BSC lined with absorbent material, loosen the cap of the primary toxin vial to allow steam penetration. Place the primary vial in a secondary biohazard sharps container with a closable lid. The sharp container is then placed into an autoclavable pan for autoclaving at 121°C and 15 psi for 60 minutes on a liquid cycle. The autoclaved sharps container is labeled as "Autoclaved Microbial Toxin for Incineration". Complete all required information on red biological waste disposal tag and affix the generator bar code sticker (See Appendix 4 for sample tag).

Glassware: Contaminated glassware can be reused after autoclaving if autoclaving is the appropriate inactivation method for the toxin used.

Solid Waste: All disposable solid waste (gloves, gauze pads, plastic ware, etc.) must be autoclaved prior to disposal if autoclaving is the appropriate inactivation method.

Chemical inactivation: Confirm that the chemicals in the original solution are compatible with the sodium hypochlorite and/or sodium hydroxide. The bleach solution should be freshly prepared and appropriate concentration of bleach and/or sodium hydroxide are used. Inside a Fumehood or BCS lined with absorbent material, open the toxin vial and place directly into the disinfectant solution. Allow the solution to stand for at least 60 minutes (check the contact time in SDS and approved biosafety protocol). The solution should be neutralized either by adding neutralizer or by leaving it for at least 24 hours before being disposed of the drain. The empty toxin vial is placed in the sharps container with a closable lid and labeled as "Chemically Deactivated Microbial Toxin for Incineration". Complete all required information on red biological waste disposal tag (Appendix 4) and affix the generator bar code sticker.

Glassware: Contaminated glassware can be reused after soaking in a mixture of 2.5 % NaOCl and 0.25N NaOH for 8 hours. Alternatively, soak in 5% bleach for 8 hours. The solution should be neutralized either by adding neutralizer or by leaving it for at least 24 hours before being disposed of the drain.

Solid Waste: All disposable solid waste (gloves, gauze pads, plastic ware, etc.) must be soaked in a mixture of 2.5 % NaOCl and 0.25N NaOH for ≥16 hours prior to disposal. Alternatively, soak in 5% bleach for ≥16 hours. The solution should be neutralized either by adding neutralizer or by leaving it for at least 24 hours before being disposed of the drain.

Incineration: If inactivation by an autoclaving or chemical method is not possible, materials will be sent for incineration. Attach the red tag and label it as "Microbial Toxin for Incineration".

Disposal

For the disposal of microbial toxin waste, Waste generators must contact ESF (604-827-5389). ESF will arrange for direct pick-up by a hazardous waste contractor.

REVIEW AND RETENTION

This Guideline is reviewed annually or whenever deemed necessary by the UBC Biosafety Committee or the UBC Biosafety Office.

APPENDIX 1: HPTA REGULATED BIOLOGICAL TOXINS

Toxin	Security Sensitive	Trigger Quantity (mg)
Aerolysin		
Alpha Toxin	Yes	5
Anthrax Roxins: Lethal Toxin and Oedema Toxin		
Bordetella Pertussis Adenylate Cyclase Toxin		
Botulinum neurotoxin	Yes	0.5
Cholera Toxin	Yes	10
Clostridium botulinum C2 and C3 toxins		
Clostridium difficile toxins A and B		
Clostridium perfringens Epsilon toxin	Yes	5
Dermonecrotic toxin		
Diptheria Toxin		
E. coli Cytotoxic Necrotizing Factor (CNF),		
Heat-labile E. coli enterotoxin (LT),		
Heat-stable E. colienterotoxin (ST),		
Cytolethal distending toxin (CLDT)		
Enteroaggregative Shiga-like toxin 1 (EAST)		
Exfoliative toxin (also called Exfoliatin)		
Exotoxin A		
Hemolysin	Yes	10
Listeriolysin O		
Pasteurella multocida toxin		
Perfringolysin O		
Pertussis toxin		
Pneumolysin		
Pyrogenic exotoxin		
Shiga-like toxin (verotoxin)	Yes	1
Shigatoxin	Yes	1
Staphylococcal enterotoxins	Yes	Type B (1) Not type B (10)
Staphylococcus aureus Toxic shock syndrome toxin	Yes	5
Streptolysin O		
Tetanolysin		
Tetanospasmin (Tetanus toxin)		

APPENDIX 2: HEAT INACTIVATION OF SELECTED TOXINS

Toxin	Steam Autoclave 1 hr. and 121°C	Dry Heat (10 min)			
		94°C	260° C	538° C	816° C
Abrin	Yes	ND	ND	ND	ND
Brevetoxin (Pb Tx-2)	No	No	No	No	Yes
Botulinum	No	No	No	No	No
Cholera toxin	Yes	ND	ND	ND	ND
Diphtheria toxin	Yes	ND	ND	ND	ND
Microcystin	No	No	Yes	Yes	Yes
Palytoxin	No	No	Yes	Yes	Yes
Pertussis toxin	Yes				
Ricin	Yes	Yes	Yes	Yes	Yes
Saxitoxin	No	No	Yes	Yes	Yes
Staphylococcal Enterotoxin	Yes	Incomplete	Incomplete	Incomplete	Incomplete
Tetrodoxin	No	No	Yes	Yes	Yes
T-2 mycotoxin	No	No	No	No	Yes
Tetanus toxin	Yes	ND	ND	ND	ND

ND: not determined from available inactivation literature

APPENDIX 3: CHEMICAL INACTIVATION OF SELECTED TOXINS

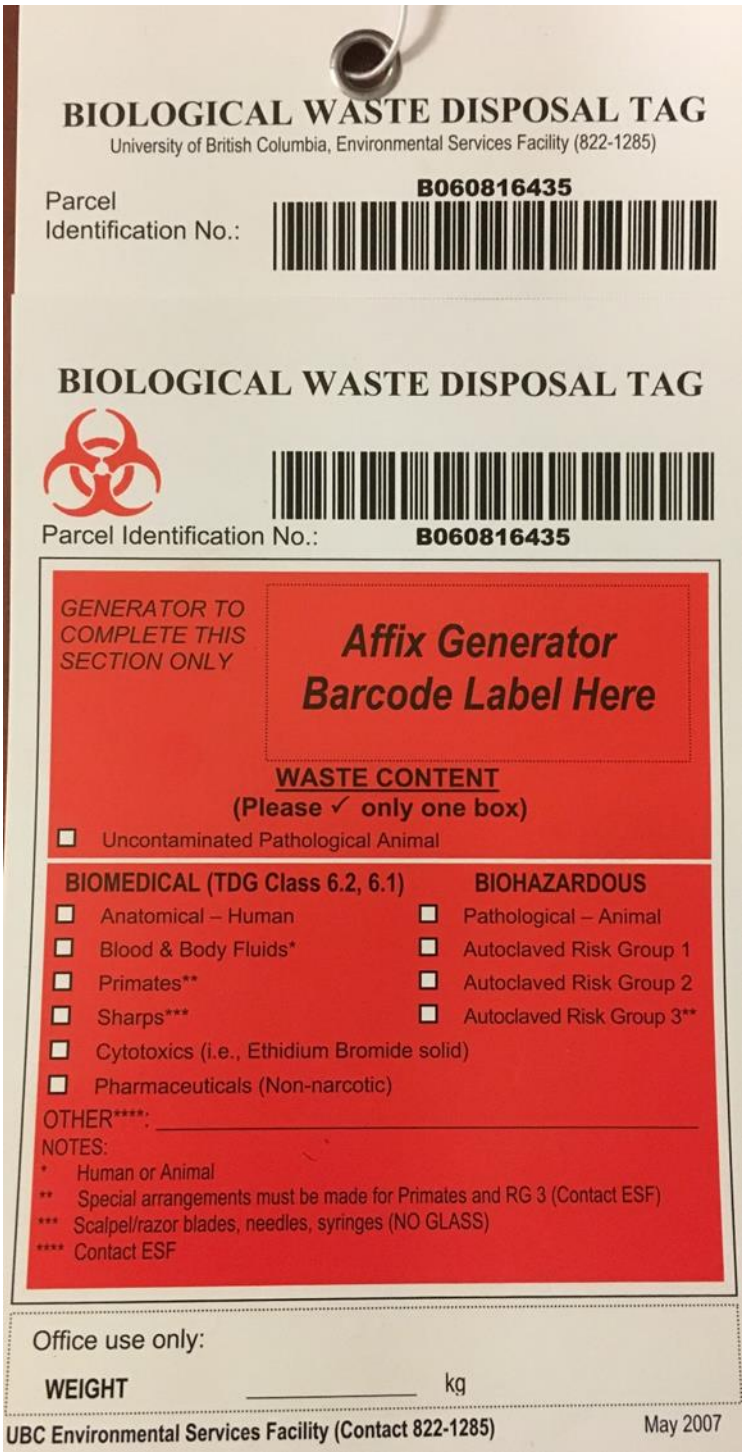
Toxin	NaOCl* 30 min	NaOH 30 min	NaOCl + NaOH 30 min	Comments
Abrin	No	No	No	Heat inactivation is recommended
Anthrax Lethal Toxin	0.50%	No	No	0.5 % NaOCl is recommended for spill cleanup.
Brevetoxin (Pb Tx-2)	2.50%	ND	ND	2.5 % NaOCl is recommended for spill cleanup.
Botulinum	0.10%	0.25N	ND	0.1 % NaOCl or .025 N NaOH for 30 min is recommended for spill cleanup.
Cholera toxin	0.50%	ND	ND	0.5 % NaOCl is recommended for spill cleanup.
Diphtheria toxin	0.50%	ND	ND	0.5 % NaOCl is recommended for spill cleanup.
Microcystin	0.50%	ND	0.25% + 0.25N	Alkalinity is the inactivation factor.
Palytoxin	0.10%	ND	0.25% + 0.25N	Alkalinity is the inactivation factor.
Pertussis toxin	0.50%	ND	ND	0.5 % NaOCl is recommended for spill cleanup.
Ricin	0.10%	ND	0.1% + 0.25N	1.0 % NaOCl is recommended for spill cleanup.
Saxitoxin	0.10%	ND	0.1% + 0.25N	1.0 % NaOCl for 30 min is recommended for spill cleanup.
Staphylococcal Enterotoxin	0.5%	0.25N	ND	SEB is heat stable. Inactivation is with 0.5% NaOCl for 15 min.

Toxin	NaOCl* 30 min	NaOH 30 min	NaOCl + NaOH 30 min	Comments
Tetrodoxin	0.50%	ND	0.1% + 0.25N	1.0 % NaOCl for 30 min is recommended for spill cleanup.
T-2 mycotoxin	2.50%	ND	0.25% + 0.25N	2.5 % NaOCl + .025 N NaOH for 4 hr is recommended for spill cleanup.
Tetanus toxin	0.50%	ND	ND	0.5 % NaOCl is recommended for spill cleanup.

* NaOCl concentrations refer to sodium hypochlorite, not household bleach. The concentration of NaOCl in household bleach is 5.25%. A dilution of 1 part household bleach in 9 part liquid is a 0.525% solution.

ND: not determined from available inactivation literature

APPENDIX 4: SAMPLE OF BIOLOGICAL WASTE DISPOSAL TAG



BIOLOGICAL WASTE DISPOSAL TAG

University of British Columbia, Environmental Services Facility (822-1285)

Parcel Identification No.: B060816435

BIOLOGICAL WASTE DISPOSAL TAG

Parcel Identification No.: B060816435

GENERATOR TO COMPLETE THIS SECTION ONLY

Affix Generator Barcode Label Here

WASTE CONTENT

(Please ✓ only one box)

☐ Uncontaminated Pathological Animal

BIOMEDICAL (TDG Class 6.2, 6.1)

☐ Anatomical – Human
☐ Blood & Body Fluids*
☐ Primates**
☐ Sharps***
☐ Cytotoxics (i.e., Ethidium Bromide solid)
☐ Pharmaceuticals (Non-narcotic)

BIOHAZARDOUS

☐ Pathological – Animal
☐ Autoclaved Risk Group 1
☐ Autoclaved Risk Group 2
☐ Autoclaved Risk Group 3**

OTHER****: _____

NOTES:
* Human or Animal
** Special arrangements must be made for Primates and RG 3 (Contact ESF)
*** Scalpel/razor blades, needles, syringes (NO GLASS)
**** Contact ESF

Office use only:

WEIGHT _____ kg

UBC Environmental Services Facility (Contact 822-1285) May 2007