Working with Toxins of Biological Origin in the Laboratory

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Definition of biological toxin

- Poisonous substances produced as by-products of microorganisms, plants or animals
- Not living organisms
- Not contagious

Resources

- CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories, 5th edition Appendix I,* Guidelines for Work With Toxins of Biological Origin (http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_appendixI.pdf)
- U of M Biosafety Manual, Section XVI, Toxins of Biological Origin (http://www.dehs.umn.edu/bio_pracprin.htm)
- MSDS (http://www.dehs.umn.edu/hazwaste_msds.htm)
- Toxin Fact Sheet (http://www.dehs.umn.edu/PDFs/Toxins.pdf)
- Toxin Guidelines (http://www.dehs.umn.edu/PDFs/Biological%20Toxin%20Guidelines.pdf)

Institutional Biosafety Committee

- IBC filing is required for working with biologically-derived toxins
- Download Biological Toxin Form from IBC web site
  http://cflegacy.research.umn.edu/ibc/download/index.cfm
- Send documents to the IBC at ibc@umn.edu
- The approved protocol is effective for 3 years with annual review

Lab Specific Standard Operating Procedures (SOPs)

The lab facilities, equipment, and procedures appropriate for work with biological toxin must reflect the intrinsic level of hazard posed by a particular toxin as well as the potential risks inherent in the operations performed. SOP template is available at http://www.dehs.umn.edu/PDFs/writingSOP.pdf

- Perform Risk Assessment
  - Identify the potential hazards associated with materials and procedures performed (i.e., exposure to biological toxin)

Lab Specific SOPs (*Continued*)

- A good reference is MSDS
  - LD_{50}
  - Toxicity Symptoms
  - Potential routes of exposure (inhalation, ingestion, dermal, parenteral, mucous membrane, etc)
  - Available antidote

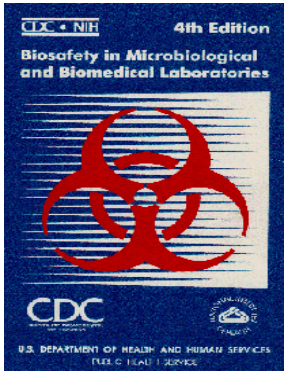
Lab Specific SOPs (*Continued*)

- Determine Exposure Risk
  - Aerosol production (centrifuge, aspiration, etc) http://www.dehs.umn.edu/PDFs/aerosol.pdf
  - Dermal exposure (e.g., T-2 mycotoxin)
  - Mucous membranes exposure
  - Ingestion
  - Parenteral (e.g., needlestick, bite)
  - Toxin work involving animals

Lab Specific SOPs (*Continued*)

- Develop Exposure Control Plan
  - Administrative Control
    - General lab safety training
      http://www.dehs.umn.edu/training_newlabsafety.htm
    - Lab specific safety training

Lab Specific SOPs (*Continued*)



- Good Work Practices
  - Routine operations with dilute toxin solutions are conducted under BSL2 conditions with the aid of PPE and appropriate engineering controls (*BMBL 5th edition, Appendix I*)

    http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_appendixI.pdf

Lab Specific SOPs (*Continued*)

- Engineering Controls with exhaust HEPA filters and charcoal filters if the material is volatile, radionucleotides combined with LMW toxin solutions
  - A certified biosafety cabinet
  - Chemical fume hood based on risk assessment
  - Centrifuge secondary containment (e.g., safety bucket with covers, sealed rotor with O-ring)

Lab Specific SOPs (*Continued*)

- Personal Protective Equipment
  - Lab gown, appropriate gloves, eye protection
  - PPE for dermally active toxins
    - ❖ Standard PPE plus full face shield, double gloves, sleeves, etc
  - PPE for dry toxins
    - ❖ Respirator, anti-static gloves, wrap-around gown

Lab Specific SOPs (*Continued*)

- Glove Selection (http://www.dehs.umn.edu/PDFs/gloves.pdf)
  - ❖ If it's absolutely necessary, when handling dry form of toxins that are electrostatic, do not wear gloves that help to generate static electricity
  - ❖ When handling toxins that are percutaneous hazards, select gloves that are known to be impervious to the toxin
  - ❖ Consider both toxin and solvent when selecting gloves and other protective clothing

Lab Specific SOPs (*Continued*)

- Biological Toxin Waste Disposal
  - Use an appropriate disinfectant for the toxin. Many toxins are susceptible to 10% bleach, 2N NaOH
    - Work surface
    - Equipment

Lab Specific SOPs (*Continued*)

- Procedures for inactivation of dry biological toxins or solution containing biological toxin, see http://www.dehs.umn.edu/bio_disposal.htm#inactivation
  - Treat with 2N NaOH for at least 1 hr
  - Other proven effective inactivating agent
- Depending upon the toxin, contaminated materials can be inactivated by autoclaving (e.g., bacterial protein toxins), see table 1 or by soaking in the appropriate decontamination solutions (e.g., LMW toxins), see table 2

Table 1. Physical Inactivation of Selected Toxins

Toxin	Steam Autoclave	Dry Heat (10 min)	Freeze-thaw	Gamma Irradiation
Botulinum neurotoxin	Yes [a]	> 100° C [b]	No [c]	Incomplete [d]
Staphylococcal Enterotoxin	Yes [e]	> 100° C; refolds [f]	No [g]	Incomplete [h]
Ricin	Yes [i]	> 100° C [i]	No [j]	Incomplete [k]
Microcystin	No [l]	> 260° C [m]	No [n]	ND
Saxitoxin	No [l]	> 260° C [m]	No [n]	ND
Palytoxin	No [l]	> 260° C [m]	No [n]	ND
Tetrodotoxin	No [l]	> 260° C [m]	No [n]	ND
T-2 mycotoxin	No [l]	> 815° C [m]	No [n]	ND
Brevetoxin (PbTx-2)	No [l]	> 815° C [m]	No [n]	ND

A Steam autoclaving should be at >=121°C for 1hr. ***CDC/NIH BMBL 5th edition, Appendix I***

http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_appendixI.pdf

Table 2. Chemical Inactivation of Selected Toxins

Toxin	NaOCl (30 min)	NaOH (30 min)	NaCOl + NaOH (30 min)	Ozone Treatment
Botulinum neurotoxin	> 0.1% [a]	> 0/25 N	ND	Yes [b]
Staphylococcal Enterotoxin	> 0.5% [c]	> 0.25 N	ND	ND
Ricin	> 1.0% [d]	ND	> 0.1% + 0.25N [e]	ND
Saxitoxin	≥ 0.1% [e]	ND	0.25% + 0.25N [e]	ND
Palytoxin	≥ 0.1% [e]	ND	0.25% + 0.25N [e]	ND
Microcystin	≥ 0.5% [e]	ND	0.25% + 0.25N [e]	ND
Tetrodotoxin	≥ 0.5% [e]	ND	0.25% + 0.25N [e]	ND
T-2 mycotoxin	≥ 2.5% [e, f]	ND	0.25% + 0.25N [e]	ND
Brevetoxin (PbTx-2)	≥ 2.5% [e, f]	ND	0.25% + 0.25N [e]	ND

Lab Specific SOPs (*Continued*)

- Develop a Lab Specific Decontamination and Spill Clean-up Plan

Decontamination & Spill Clean-up Plan Template

http://www.dehs.umn.edu/Docs/DecontaminationTemplate.doc

General Practices

- No eating or drinking in the lab
- Cover work surfaces with disposable plastic-backed absorbent pad & dispose of as biohazardous waste
- Work surfaces are decontaminated at least once a day using methods known to be effective against toxin and after any spill of toxin (e.g., 10% bleach)
- Remove gloves, lab coat, and wash hands before leaving the lab
- Safe handling of sharps

Special Practices

- When toxins are in use, the room should be posted: ”Toxin in Use-Authorized Personnel Only”
- All procedures are performed carefully to minimize the creation of splashes or aerosols
- Training specific to the toxins used should be required and documented for lab personnel working with toxins. Training record template is available at http://www.dehs.umn.edu/Docs/SafetyTrainingRecord.doc

Special Practices (*Continued*)

- Security of toxins
  - Maintain an accurate inventory of purified toxins
  - Toxins must be secured whenever unattended (e.g., locked room, locked freezer, locked box)
  - For the select agent toxins, the lab door must be posted with “Biologically-derived Toxins”. Federal select agent list is available at http://www.dehs.umn.edu/bio_pracprin_sa_list.htm

Special Practices (*Continued*)

- Routine operations with dilute toxin solutions are conducted under BSL2 containment
- If centrifuge secondary containment is not available, wait at least 10 minutes after the run to allow aerosol to settle before opening centrifuge. For centrifuge safety, see http://www.dehs.umn.edu/PDFs/centrifuge.pdf
- Before containers are removed from bench top or BSC, the exterior of closed primary containers should be decontaminated and placed in 2nd containers

Special Practices (*Continued*)

- If toxins are moved between labs, always use a leak-proof sealed primary container within a leak-proof sealed secondary container. Place absorbent material between primary and secondary containers
- When vacuum lines are used with systems containing toxins, they should be protected with a HEPA filter and traps to prevent entry of toxins into the lines

Special Practices (*Continued*)

The flask A is used to collect fluids into a suitable decontamination solution; flask B containing appropriate decontamination solution serves as a fluid overflow collection vessel. A glass sparger in flask B minimizes splatter. An in-line HEPA filter (C) is used to protect the vacuum system (D) from aerosolized materials.

Special Practices (*Continued*)

- Solid or dry toxin
  - Very dangerous, contamination of work surfaces is very likely
  - Whenever possible, reconstitute entire vial of powdered toxin by injecting solvent through septum
  - Unavoidable operations with dry toxin should be undertaken with appropriate respiratory protection and in a ducted Class II or Class III BSC

Special Practices (*Continued*)

- The presence of two knowledgeable individuals is required in the lab in the following situations:
  - Manipulating dry forms of toxins
  - Operations involving intentional aerosol formation
  - The use of needles in conjunction with amounts of toxin estimated to be lethal for humans. For sharps safety see http://www.dehs.umn.edu/bio_pracprin_su_ss.htm

Special Practices (*Continued*)

- Select Agent Toxins
  - Select agent toxins quantities exceeding certain amounts are subject to federal select agent registration and regulations
    http://www.dehs.umn.edu/bio_pracprin_sa_reg.htm
  - Labs using or possessing exempt quantities of select agent toxins must follow U of M procedures for working with exempt quantities of select agents
    http://www.dehs.umn.edu/bio_pracprin_sa_exempt.htm

Questions