

Handling and Disposal of Biotoxins

A biotoxin is a poisonous substance that is a specific product of the metabolic activities of a living organism (plant, animal, fungus, bacteria). Unlike most other biohazards, biotoxins do not replicate and, in some senses, are more analogous to chemical toxins. Proper handling and disposal of biotoxins pose special challenges but yet are vital steps toward the protection of laboratory and service personnel.

Solubilization of Biotoxins

Biotoxins are frequently lyophilized or otherwise concentrated into the powdered form as the final preparation stage. Commercial preparations typically are shipped in crimped vials that are topped by a rubber stopper, while the individual laboratory may package and store the powder in vials or microcentrifuge tubes. Solubilizing the powder to formulate a stock solution presents a risk to the researcher because of the possibility of dispersal of the powder into the air. The protocols below are designed to minimize the risk of personnel exposure and environmental contamination.

Biotoxin stock solution preparation in crimped vials:

- Work in a chemical fume cabinet.
- Gloves should be worn that are resistant to the diluent.
- Add diluent through rubber stopper via syringe and needle (Luer Lock or one-piece needle and syringe).
- If necessary, allow pressure differential within the vial to dissipate by withdrawing the needle above the meniscus and allowing the syringe plunger to be displaced.
- The needle and syringe can be used to further assist in the solubilization by alternately withdrawing and expelling the solution through the stopper. Once the liquid has been added to the vial and the powder has been wetted, the needle/syringe should be removed and disposed. The needle/syringe should not be used for handling/dispersing/aliquoting the toxin-containing solution.

Biotoxin stock solution preparation in microcentrifuge tubes:

- These containers are not meant to be penetrated by a needle-syringe. Opening the container top is apt to lead to powder dispersal from pressure differential or static electricity. Spraying static guard on gloves is highly recommended. Two examples are Kensington Dust Guardian® Antistatic Spray Cleaner and KleenMaster Brilliantize Cleaner and Polish.
- Gloves should be worn that are resistant to the diluent; in addition to the usual PPE, full face protection should also be worn.
- Open the container slowly behind a Plexiglas shield, preferably in an isolated portion of the laboratory with limited air-flow. It is best not to open the container completely (usually a crack sufficient for addition of the solvent/diluent will work).
- After addition of the diluent, mixing or vortexing should be done in a chemical fume hood. Once the powder is wetted completely, then the tube can be opened carefully without concern about air dispersal. Note: when possible tubes should be centrifuged briefly to remove liquid drops from caps/lids.



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Disposal Procedures for Biotoxins

The method of disposal is dependent on the chemical composition of the biotoxin. Some of the proteinaceous biotoxins can be effectively inactivated by exposure to 10% bleach for at least one hour of contact time or by autoclaving at 121° C and 15 psi, for one hour. Examples of proteinaceous biotoxins that can be disposed of by these methods are Staphylococcus enterotoxin, ricin, and anthrax lethal toxin¹. Contact the Biological Safety Section [via e-mail](mailto:bss@illinois.edu) at bss@illinois.edu or 333-2755 if you have disposal questions for other proteinaceous biotoxins.

The inactivation of non-proteinaceous biotoxins is less clear. There is conflicting evidence as to which methods are most effective. Examples of non-proteinaceous biotoxins are aflatoxin, T-2 toxin, and some venoms and marine toxins. The following disposal instructions have been developed to ensure all the non-proteinaceous biotoxin wastes are disposed in a manner that is consistent and safe for all personnel involved. Questions regarding these procedures should be directed to the Chemical Safety Section, Division of Research Safety at 333-2755 or [via e-mail](mailto:bss@illinois.edu) at bss@illinois.edu.

Note: These instructions apply only to biotoxins that are not regulated as select agents. If you are working with a biotoxin that is regulated as a select agent, contact the Biological Safety Section [via e-mail](mailto:bss@illinois.edu) at bss@illinois.edu or 333-2755. To determine if you are working with a biotoxin that is regulated as a select agent, refer to the list at the end of this document.

Solid waste containing non-proteinaceous biotoxins (includes biotoxin contaminated debris)

(Debris includes disposable items such as gloves, labcoats, absorbent paper, plastic pipette tips and empty containers)

- Do not add bleach or any other chemical to deactivate.
- Place the contaminated items directly in a puncture-resistant bag, no larger than 10 gal in size (small trash can size) and keep weight below 20 lbs. The bag must be labeled with the contents – e.g. aflatoxin contaminated debris.
- Do not use biohazard bags or anything marked with a biohazard symbol. You will be required to repackage the waste if there are any biohazard symbols visible or covered.

Some labs have found it useful to place a bag in a container that has a lid to minimize exposure. The container holding the bag should be clearly marked so that custodial staff does not mistake it for regular trash.

- When full, seal bag and confirm the label on the bag is accurate.
- Submit a chemical waste pickup request form. Indicate the maximum amount of toxin present in the waste on the form (this is to verify that quantities do not qualify for regulation as select agents, e.g. tetrodotoxin <100mg.). Chemical waste pickup request forms can be obtained online at: www.drs.illinois.edu/regwaste/chem/index.aspx
- You will receive a label for your waste in 7-12 working days after submitting your form. Apply the label to the waste bag and have it ready for pickup on the date the notice sent with your label(s) indicates.



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Liquid waste containing non-proteinaceous biotoxins

- Do not add bleach or any other chemical to deactivate.
- Collect waste in glass or plastic containers with screw cap lids.
- Label containers as to the contents – list all chemicals in the liquid waste including the biotoxin. Do not label with a biohazard symbol. You will be required to repackage the waste if there are any biohazard symbols visible or covered.
- Submit a chemical waste pickup request form. Indicate the maximum amount of toxin present in the waste on the form (this is to verify that quantities do not qualify for regulation as select agents, e.g. tetrodotoxin <100mg.). Chemical waste pickup request forms can be obtained online at: www.drs.illinois.edu/regwaste/chem/index.aspx. Same as above.

Biotoxins that are regulated as select agents

A biotoxin is regulated as a select agent only if the aggregate amount of the biotoxin under the control of a principal investigator exceeds the amount listed in parenthesis for each toxin².

- Abrin (100mg)
- Botulinum Neurotoxins (0.5 mg)
- *Clostridium perfringens* epsilon toxin (100 mg)
- Conotoxins (100 mg)
- Diacetoxyscirpenol (1000 mg)
- Ricin (100 mg)
- Saxitoxin (100 mg)
- Shiga-like ribosome inactivating proteins (100 mg)
- Shigatoxin (100 mg)
- Staphylococcal enterotoxins (5 mg)
- Tetrodotoxin (100 mg)
- T-2 toxin (1000 mg)

References

¹Wannemacher R.W. 1989. Procedures for Inactivation and Safety Containment of Toxins. Proc. Symposium on Agents of Biological Origin, U.S. Army Research, Dev. and Engineering Center, Aberdeen proving Ground, MD. pp. 115-122

² <http://www.cdc.gov/od/sap/sap/toxinamt.htm>

Questions

For more information, contact the Division of Research Safety, Biological Safety Section (333-2755 or [via e-mail at bss@illinois.edu](mailto:bss@illinois.edu)) or visit our web site: <http://www.drs.illinois.edu/bss/>.

Other Biosafety Fact Sheets are available from the Biological Safety Section at our web site: <http://www.drs.illinois.edu/bss/factsheets/>.

