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Requirements for Work with Toxins of Biological Origin

(Adapted from CDC Biosafety in Microbiological and Biomedical Laboratories, Appendix I, 5th ed, Dec 2009)

Each Principle Investigator (PI) is responsible for the appropriate risk assessment for each specific laboratory operation and any biological, chemical or radioactive hazardous material in use in their laboratory, as well as the on-the-job training of all research staff in the necessary SOPs.

These recommendations and the listed references should be used by each Principle Investigator (PI) to create laboratory Standard Operating Procedures (SOPs) for the toxins used, including the known or suspected health hazards, safety steps and PPE equipment for the laboratory manipulation of toxins, segregation of wastes, treatment of wastes and final disposal of all biological toxins, and emergency procedures (e.g. for exposure incidents, spills, etc).

Introduction

Biological toxins[1] comprise a broad range of poisons, predominantly of natural origin from microorganisms or other living organisms, but increasingly possible to generate with modern synthetic methods. Biological toxins may cause death or severe incapacitation at relatively low exposure levels.

The deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates, with an LD_{50} of < 100 nanograms per kilograms body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, *Shigella dysenteriae* neurotoxin) is specifically regulated by the NIH Office of Biotechnology Activities, *NIH Guidelines for Research Involving recombinant DNA,* Section III-B-1. Such experiments require NIH/OBA approval and local Institutional Biosafety Committee approval before initiation.

Laboratory safety principles are summarized here for biological toxins currently in use at UCDenver, including those regulated as "Select Agent Toxins". Additional details for safe practices for researchers should be found in the Material Safety Data Sheet (MSDS) provided by the vendor or source of the toxin.

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[1] Poisonous substances produced by certain bacteria, fungi, protozoa, plants, reptiles, amphibians, fish, echinoderms (spiny urchins and starfish), mollusks, and insects.

Additional assistance in creating these SOPs is available from the UCDenver Dept. of Environmental Health and Safety. Contact us at 303-724-0345.

General Considerations for Toxin Use

Laboratory work with most toxins, in amounts routinely employed in the biomedical sciences, can be performed safely with minimal risk to the worker and negligible risk to the surrounding community when appropriate procedures, PPE and containment measures are implemented
Toxins do not replicate, are not infectious, and are difficult to transmit mechanically or manually from person to person. Many commonly employed toxins have very low volatility and, especially in the case of protein toxins, are relatively unstable in the environment; these characteristics further limit the spread of toxins.

Degree of toxicity, when known, is generally expressed in terms of "lethal dose" in a specific animal species, reported as LD_{50}, per kilograms of body weight that will kill 50% of the test animals via a known route of exposure (e.g. oral, IP, etc)

NOTE: Toxicity testing in animal species is not always readily extrapolated to human exposures and in the case of certain toxins, exposures to humans may present as far more toxic than in other species.

The major risks with bench research and/or animal experiments are accidental exposure by direct contamination of mouth, eyes or other mucous membranes; by inadvertent aerosol generation; and by needle-sticks or other accidents that may compromise the normal barrier of the skin.

All toxins should be considered hazardous by inhalation. Aerosols are generally created when working with dry forms of toxins or upon mechanical agitation, such as vortex mixing is used in laboratory preparation of materials.

Toxins can be handled using established general guidelines for toxic or highly-toxic chemicals with the incorporation of additional safety and security measures based upon a risk assessment for each specific laboratory operation.

Laboratory Training and Planning of Experiments

Each laboratory worker must be trained with respect to the toxins to be used, with special emphasis on the nature of the practical hazards associated with laboratory operations.

Training must include:

- how to safely reconstitute toxins from dry powder or lyophilized state;
- any hazards associated with the liquid(s) used to reconstitute toxins (e.g. skin absorption hazards of DMSO);
- how to handle transfers of liquids containing toxin;
- how to properly segregate waste solutions and contaminated materials or equipment into the correct waste stream; and
- how to decontaminate work areas after routine operations, as well as after accidental spills;

- how to appropriately render first aid, seek medical care and report incidents/injuries or spills involving toxins.

Each worker must be reliable and sufficiently adept at all required manipulations before being permitted to work with toxins. A risk assessment should be conducted to develop safe operating procedures before undertaking laboratory operations with toxins; suggested "pre-operational checklists" for working with toxins are available.

For complex operations, it is recommended that new workers undergo supervised practice runs in which the exact laboratory procedures to be undertaken are rehearsed without active toxin. If toxins and infectious agents are used together, then both must be considered when containment equipment is selected and safety procedures are developed. Likewise, safety practices must be considered for toxin work involving animals and SOPs developed for work within the vivaria.

Each laboratory that uses toxins should develop a specific Chemical Hygiene Plan. The National Research Council has provided a review of prudent practices[2] when handling toxic and highly toxic chemicals, including the development of chemical hygiene plans and guidelines for compliance with regulations governing occupational safety and health, hazard communication, and environmental protection.

An inventory control system should be in place to account for toxin use and disposition. If toxins are stored in the laboratory, containers should be sealed, labeled, and secured to ensure restricted access; refrigerators and other storage containers should be clearly labeled and provide contact information for trained, responsible laboratory staff.

Laboratory work with toxins should be done only in designated rooms with controlled access and at pre-determined bench areas. When toxins are in use, the room should be clearly posted: "Toxins in Use—Authorized Personnel Only.

Unrelated and nonessential work should be restricted from areas where stock solutions of toxin or organisms producing toxin are used. Visitors or other untrained personnel granted laboratory access must be monitored and protected from inadvertently using laboratory equipment used to manipulate the toxin or organism.

Safety Equipment and Containment

Routine operations with dilute toxin solutions can be safely conducted with the aid of engineered controls, such as a chemical fume hood, and appropriate personal protective equipment (PPE)

Engineering controls should be selected according to the risk assessment for each specific toxin operation. A certified BSC or chemical fume hood will suffice for routine operations with most protein toxins.

[2] Prudent Practices in the Laboratory is available from the National Academies Press at http://www.nap.edu/openbook.php?isbn=0309052297

Low molecular weight toxin solutions, or work involving volatile chemicals or radionucleotides combined with toxin solutions, may require the use of a charcoal-based hood filter in addition to HEPA filtration.

If additional respiratory protection is warranted, please contact the EHS-Industrial Hygiene staff, 303-724-0242, for risk assessment, respirator selection, training and fit-testing.

All work with toxins should be conducted within the operationally effective zone of the hood or BSC, and each user should verify the inward airflow before initiating work. When using an open-fronted fume hood or BSC, workers should wear suitable laboratory PPE to protect the hands and arms, such as laboratory coats, smocks, or coveralls and disposable gloves.

When working with toxins that pose direct percutaneous hazards, special care must be taken to select gloves that are impervious to the toxin and the diluents or solvents employed.

NOTE: Latex gloves are *not* appropriate for work with toxins and many diluents and solvents since they provide no protection against permeability. Gloves should be nitrile or better. When working with toxins or diluents or solvents that are dermally active, a full-face shield and additional arm and hand protection (coverings) are highly recommended.

For additional guidance and information on glove suitability consult the EHS Industrial Hygiene staff, 303-724-0242.

When conducting liquid transfers and other operations that pose a potential splash or droplet hazard in an open-fronted hood or BSC, workers should wear safety glasses and disposable facemask, or a face shield.

Toxin should be removed from the hood or BSC only after the exterior of the closed primary container has been decontaminated and placed in a clean secondary container. Toxin solutions, especially concentrated stock solutions, should be transported in leak/spill-proof secondary containers.

The interior of the hood or BSC should be decontaminated periodically, for example, at the end of a series of related experiments. Until thoroughly decontaminated, the hood or BSC should be posted to indicate that toxins have been used, and access should remain restricted.

Selected operations with toxins may require modified BSL-3 practices and procedures**.** The determination to use BSL-3 is made in consultation with available safety staff and is based upon a risk assessment that considers the variables of each specific laboratory operation, especially the toxin under study, the physical state of the toxin (solution or dry form), the total amount of toxin used relative to the estimated human lethal dose, the volume of the material manipulated, the methodology, and any human or equipment performance limitations.

Inadvertently Creating Toxin Aerosols

Emphasis must be placed on evaluating and modifying experimental procedures to eliminate the possibility of inadvertent generation of toxin aerosols. Pressurized tubes or other containers holding toxins should be opened in a BSC, chemical fume hood, or other ventilated enclosure.

Operations that expose toxin solutions to vacuum or pressure, for example sterilization of toxin solutions by membrane filtration, should always be handled in this manner, and the operator should also use appropriate respiratory protection. If vacuum lines are used with toxin, they should be protected with a HEPA filter to prevent entry of toxins into the line.

Centrifugation of cultures or materials potentially containing toxins should only be performed using sealed, thick-walled tubes in safety centrifuge cups or sealed rotors. The outside surfaces of containers and rotors should be routinely cleaned before each use to prevent contamination that may generate an aerosol. After centrifugation, the entire rotor assembly should be taken from the centrifuge to a BSC or chemical fume hood to open it and remove materials.

Mechanical Injuries

Accidental needle-sticks or mechanical injury from “sharps” such as glass or metal implements pose a well-known risk to laboratory workers, and the consequences may be catastrophic for operations involving toxins in amounts that exceed a human lethal dose.

Only workers trained and experienced in handling animals should be permitted to conduct operations involving injection of toxin solutions using hollow-bore needles. Discarded needles/syringes and other sharps should be placed directly into properly labeled, puncture-resistant sharps containers, and segregated for waste disposal as soon as is practical.

Plastic should be substituted for glassware for handling toxin solutions wherever practical to minimize the risk of cuts or abrasions from contaminated surfaces. Thin-walled glass equipment should be completely avoided. Glass Pasteur pipettes are particularly dangerous for transferring toxin solutions and should be replaced with disposable plastic pipettes. Glass chromatography columns under pressure must be enclosed within a plastic water jacket or other secondary container.

Additional Precautions

Experiments should be planned to eliminate or minimize work with dry toxin (e.g., freeze-dried preparations). Unavoidable operations with dry toxin should only be undertaken with appropriate engineering controls, PPE and respiratory protection. Dry toxin can be manipulated using a Class III BSC, or with the use of secondary containment such as a disposable glove bag or glove box within a hood or Class II BSC. “Static-free” disposable gloves should be worn when working with dry forms of toxins that are subject to spread by electrostatic dispersal.

In specialized laboratories, the intentional, controlled generation of aerosols from toxin solutions may be undertaken to test antidotes or vaccines in experimental animals. These are extremely hazardous operations that should only be conducted after extensive validation of equipment and personnel, using non-toxic simulants.

For high-risk operations involving dry forms of toxins, intentional aerosol formation, or the use of hollow-bore needles in conjunction with amounts of toxin estimated to be lethal for humans, consideration should be given to requiring the presence of at least two knowledgeable individuals at all times in the laboratory.

Decontamination and Spills

Toxin stability varies considerably outside of physiological conditions depending upon the temperature, pH, ionic strength, availability of co-factors and other characteristics of the surrounding matrix. Literature values for dry heat inactivation of toxins can be misleading due to variations in experimental conditions, matrix composition, and experimental criteria for assessing toxin activity.

Moreover, inactivation is not always a linear function of heating time; some protein toxins possess a capacity to re-fold and partially reverse inactivation caused by heating. In addition. The conditions for denaturizing toxins in aqueous solutions are not necessarily applicable for inactivating dry, powdered toxin preparations.

General guidelines for laboratory decontamination of selected toxins are summarized in the CDC BMBL, but inactivation procedures should not be assumed to be 100% effective without validation using specific toxin bioassays.

Many toxins are susceptible to inactivation with dilute sodium hydroxide (NaOH) at concentrations of 0.1-0.25N, and/or sodium hypochlorite (NaOCl) bleach solutions at concentrations of 0.1-0.5% (w/v). Use freshly prepared bleach solutions for decontamination; undiluted, commercially available bleach solutions typically contain 3-6% (w/v) NaOCl.

Depending upon the toxin, contaminated materials and toxin waste solutions can be inactivated by incineration or extensive autoclaving, or by soaking in suitable decontamination solutions.

All disposable material used for toxin work should be placed in secondary containers, segregated into the correct waste stream and disposed of as chemical hazardous wastes.

Contaminated or potentially contaminated protective clothing and equipment should be decontaminated using suitable chemical methods or autoclaving before removal from the laboratory for disposal, cleaning or repair. If decontamination is impracticable, materials should be disposed of as chemical hazardous waste.

In the event of a spill, avoid splashes or generating aerosols during cleanup by covering the spill with paper towels or other disposable, absorbent material. Apply an appropriate decontamination solution to the spill, beginning at the perimeter and working towards the center, and allow sufficient contact time to completely inactivate the toxin.

Large-scale decontamination is not covered explicitly here. Consult the EHS Chemical Hazardous Materials unit (303-724-0344) if there is a need for more extensive clean-up efforts.

Select Agent Toxins

Due diligence must be applied in shipment, manipulation, or storage of any amount of toxin.

There are specific regulatory requirements for working with toxins designated as a "Select Agent" by the DHHS and/or the USDA.

Select Agent toxins require, at a minimum, registration with the UCDenver Institutional Biosafety Committee, and depending upon the quantities on hand may require further registration and approval of the US CDC and/or USDA for possession, use, storage and/or transfer.

Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of the agent may require a permit from USDA/ APHIS/VS.

A US Department of Commerce permit may be required for the export of the agent to another country or to allow for the sharing of information or technology with international students and scholars in your laboratory.

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