PERTUSSIS TOXIN EXPOSURE/INJURY RESPONSE PROTOCOL

Organism or Agent: Pertussis Toxin
Exposure Risk: Multiple Endocrine/Metabolic Effects
Needlestick Exposure Hotline Pager: 415/353-7842 (353-STIC) (Available 24 hours)
UCSF Occupational Health Services: 415/885-7580 (Available during work hours)
Office of Environment, Health & Safety: 415/476-1300 (Available during work hours)
415/476-1414 or 9-911 (In case of emergency, available 24 hours)
EH&S Public Health Officer: 415/514-3531

PROTOCOL SUMMARY

In the event of an accidental exposure or injury, the protocol is as follows:

1. Modes of Exposure:
   a. Skin puncture or injection
   b. Ingestion
   c. Contact with mucous membranes (eyes, nose, mouth)
   d. Contact with non-intact skin
   e. Exposure to aerosols
   f. Respiratory exposure from inhalation of toxin

2. First Aid:
   a. Skin Exposure, immediately go to the sink and thoroughly wash the skin with soap and water.
      Decontaminate any exposed skin surfaces with an antiseptic scrub solution.
   b. Skin Wound, immediately go to the sink and thoroughly wash the wound with soap and water
      and pat dry.
   c. Splash to Eye(s), Nose or Mouth, immediately flush the area with running water for at least 5-
      10 minutes.
   d. Splash Affecting Garments, remove garments that may have become soiled or contaminated and
      place them in a double red plastic bag.

3. Treatment:
   a. In the event of an acute injury or exposure resulting from a laboratory incident, the injured
      employee/student should report to the Emergency Department for medical treatment. The injured
      individual must take a copy of this entire protocol document to the Emergency Department,
      including information regarding the specific toxin subtypes associated with exposure.
   b. In the event of an exposure, with or without an injury, call the Needlestick Exposure Hotline in
      order to get access to medical care for the exposure. The needlestick exposure hotline responder
      will provide guidance to the injured individual on necessary medical treatment and post exposure
      follow-up.

4. Follow-up is needed in the event of any Laboratory Exposure:
   a. After first aid has been administered, immediately inform your supervisor of the exposure.
   b. In the event of a large spill in a secure area, leave the area and secure the lab to prevent entry of
      other personnel, and possible secondary exposures. In the event of a spill in a non-secure area,
      contact the emergency response team (9-911) for clean-up.
c. Contact UCSF Occupational Health Services after first aid /Emergency Department observation is complete, for follow-up care.
d. Contact the Biosafety Officer at 415/514-2824 to report the injury or exposure.
1. **WORKER’S RESPONSIBILITIES (Employee/Student Initial Self-Care)**
   a. **First Aid:** Perform recommended first aid and decontamination according to the posted instructions.

   b. **Treatment:** i. In the event of an exposure or injury resulting from a laboratory incident, the exposed individual should report to the Emergency Department (ED) for acute medical treatment. The employee should bring a copy of this protocol, and should be prepared to inform the ED physician of the exact types of toxin to which he/she was exposed. ii. In the event of an exposure, with or without an injury, call the Needlestick Exposure Hotline in order to get access to medical care for the exposure and evaluation for possible post exposure prophylaxis.

   c. **Access to Needlestick Hotline:** Immediately call the Needlestick Exposure Hotline in the event of an exposure. Dial 415/353-7842. However, the first priority should be reporting to the nearest emergency room with a copy of this protocol.

   d. **Reporting:** Inform your laboratory supervisor/principal investigator of the exposure. Within 24 hours, report the injury to the UCSF Human Resources Disability Management Services (HR DMS) Office on the Employee’s Incident Report (EIR) form, available here: [http://or.ucsf.edu/ehs/8969-DSY/version/default/part/4/data/](http://or.ucsf.edu/ehs/8969-DSY/version/default/part/4/data/)

   e. **Secure the laboratory:** Identify the equipment involved in the exposure and the mechanism of exposure. Make sure that the laboratory area has been secured and that notification of contamination has been posted to prevent other individuals from entering the area.

   f. **Follow-up:** Contact Occupational Health Services (OHS) at 415/885-7580 for any needed follow-up care.

2. **SUPERVISOR’S RESPONSIBILITIES**
   a. **First Aid and Decontamination:** Verify that the worker has washed and decontaminated himself/herself. Ensure that appropriate medical treatment has been received.

   b. **Secure the laboratory:** Confirm that the laboratory area has been secured and that notification of contamination has been posted to prevent other individuals from entering the area.

   c. **Laboratory clean-up (as needed):** Contact the Office of Environmental Health & Safety (OEH&S) through the UC Police Department Emergency Dispatch (from a campus telephone 9-911, from a non-campus phone 415/476-1414).

   d. **Report the exposure:** Call the Public Health Officer during regular business hours to discuss the exposure. A report summarizing any suspected pertussis toxin exposure needs to be submitted to the Biosafety Committee by the Principal Investigator (PI). The report must include the following:
      - A brief description of the exposure event, a description of the area involved, and the extent of employee exposure
      - If applicable, specification of the amount of toxic material released, time involved, and explanation of procedures used to determine the amount involved
      - Corrective action taken to prevent the re-occurrence of the incident
      - Decontamination procedures
e. **Follow-Up:** Confirm that the worker has called for an appointment at the UCSF Occupational Health Clinic.

f. **Report the Injury:** Within 24 hours, report the injury to the UCSF Human Resources Disability Management Services (HR DMS) Office on the Supervisor’s Report of Injury (SRI) form, available here: [http://ucsfhr.ucsf.edu/dismgmt/forms/workcomp/claim/SRI.pdf](http://ucsfhr.ucsf.edu/dismgmt/forms/workcomp/claim/SRI.pdf)
MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: Pertussis toxin from Bordetella pertussis

SYNONYM OR CROSS REFERENCE: Pertussis toxin

CHARACTERISTICS: Pertussis toxin (PT) is a protein-based exotoxin produced by the bacterium Bordetella pertussis, which causes whooping cough.

SECTION II - HEALTH HAZARD

PATHOGENICITY: Pertussis infections are characterized by an acute respiratory disease with three stages: a catarrhal stage with an irritating cough, lasts 1 to 2 weeks; a paroxysmal stage characterized by violent coughs followed by a high pitched inspiratory whoop, lasts 2 to 6 weeks; a convalescent stage where the cough gradually decreases in frequency and severity, lasts several weeks; 75% of deaths are among infants; parapertussis is similar but milder, occurs in school-age children and is seen less frequently.

EPIDEMIOLOGY: Infections from Bordetella pertussis are common in children worldwide; decline in incidence and mortality following immunization and where good nutrition and medical care are available; in unimmunized populations with malnutrition and multiple infections, pertussis is among the most lethal infant diseases.

HOST RANGE: Humans

INFECTIONOUS DOSE: Unknown

MODE OF TRANSMISSION: Highly toxic by inhalation, Toxic by ingestion, Toxic by skin absorption.

SECTION III - DISSEMINATION

RESERVOIR: Humans

ZOOONOSIS: None

VECTORS: None
SECTION IV - MEDICAL

SURVEILLANCE: Monitor for symptoms.

FIRST AID/TREATMENT: Consult a physician. Show this safety data sheet to the doctor in attendance. Move out of dangerous area. If inhaled: If breathed in, move person into fresh air. If not breathing give artificial respiration. Consult a physician. In case of skin contact: Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician. In case of eye contact: Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician. If swallowed: Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

IMMUNIZATION: Children receive 5 doses of DTaP, one dose at each of the following ages: 2, 4, 6, and 15-18 months and 4-6 years. Adolescents 11-18 years of age (preferably at age 11-12 years) and adults 19 through 64 years of age receive a single dose of Tdap (per current CDC Recommendations).

SECTION V - LABORATORY HAZARDS

LABORATORY-ACQUIRED INFECTIONS: Rare source of infections; one case in a worker who had aerated liquid cultures for vaccine preparation; possible infection in 8 people who worked in building where research on vaccine was being done (organism was recovered); similar incident reported in a large university research facility which resulted in 2 possible infections.

 SOURCES/SPECIMENS: Nasopharyngeal swabs and secretions.

PRIMARY HAZARDS: Direct contact of mucous membranes and inhalation of infectious aerosols and droplets; accidental parenteral inoculation; ingestion.

SPECIAL HAZARDS: None

SECTION VI - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices, containment equipment and facilities for all activities involving known or potentially infected clinical materials or cultures; animal biosafety level 2 facilities for studies utilizing infected laboratory animals. Work likely to generate aerosols should be carried out in a biosafety cabinet.

PROTECTIVE CLOTHING: Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection: Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N99 (US) or type P2 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection: Handle with gloves.

Eye protection: Safety glasses
Skin and body protection: Choose body protection according to the amount and concentration of the dangerous substance at the work place.

SECTION VII - HANDLING INFORMATION

SPILLS: Wear respiratory protection. Avoid dust formation. Avoid breathing dust. Ensure adequate ventilation. Evacuate personnel to safe areas. Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

DISPOSAL: Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

STORAGE: In sealed containers that are appropriately labeled. Store in a secure location.

PRINCIPAL INVESTIGATOR (PI) RESPONSIBILITIES

a. The PI will ensure that all lab personnel are trained in the use of safe laboratory procedures to prevent accidental exposure before assignment to any laboratory where pertussis toxin is used.

b. The PI will carefully explain the necessity of immunization with Tdap (only one dose of Tdap is currently recommended for adults). The PI will ensure that all laboratory workers are offered immunization against pertussis. Employees may choose to receive the Tdap immunization, or sign a declination form. Documentation of immunization or declination must be provided to the Public Health Office via Box 0942.

c. The PI may request assistance from UCSF OEH&S in providing information about safe laboratory procedures and the importance of immunization with Tdap. For assistance, the PI should call the UCSF Public Health Officer or Biosafety Officer.

d. The PI must ensure that all researchers who will be working with pertussis toxin have read the entire protocol. The PI will also ensure that the protocol will be reviewed on a yearly basis by all laboratory workers.

e. If working with Bordetella pertussis, and not simply the toxin, the PI shall be aware of the provisions of the California Aerosol Transmissible Disease Standard, since Bordetella pertussis is a covered entity. For information, please refer to the California Code of Regulations: [http://www.dir.ca.gov/Title8/5199.html](http://www.dir.ca.gov/Title8/5199.html).

f. The PI shall ensure that any known exposure is reported to the Biosafety officer and to the San Francisco Department of Public Health.
I. RISKS IN LABORATORY WORKERS/CLINICAL SUMMARY

Bordetella pertussis

*B. pertussis* is a fastidious, gram-negative bacterium requiring special media for isolation. *B. pertussis* produces multiple antigenic and biologically active products including:

- Pertussis toxin
- Filamentous hemagglutinin (FHA)
- Agglutinogens
- Adenylate cyclase
- Periactin
- Tracheal cytotoxin

These products are responsible for the clinical features of pertussis, and an immune response to one or more produces immunity following infection.

Pathogenesis

Pertussis is primarily a toxin-mediated disease. The bacteria attach to the cilia of the respiratory epithelial cells, produce toxins that paralyze the cilia, and cause inflammation of the respiratory tract, which interferes with the clearing of pulmonary secretions. Until recently, it was thought that *B. pertussis* did not invade the tissues; however, recent studies have suggested that the bacteria are present in alveolar macrophages.

From:  http://www.cdc.gov/pertussis/clinical/disease-specifics.html

- Pertussis, or whooping cough, is a disease caused by *B. pertussis*, a gram-negative bacterium, which adheres to the cilia of the upper respiratory tract of humans, colonizes this tissue, and releases a number of virulence factors responsible for the local and systemic damages associated with the disease (paroxysmal cough, accompanied by whoops, vomiting, cyanosis and apnea). Vaccination is the only way to control pertussis. Mass vaccination using killed bacteria (cellular vaccine) was introduced in the 1950s and reduced by 99% the incidence of the disease in infants. In the 1980s, the use of this vaccine in developed countries decreased dramatically because of concerns of potential side effects associated with vaccination. This stimulated the search for an acellular vaccine that retained efficacy but was less reactive than the whole-cell vaccine. Several molecules produced by *B. pertussis* were identified as candidates for inclusion in acellular vaccine against whooping cough. This included molecules involved in the adhesion of the bacteria to the eukaryotic cells and to the cilia of the upper respiratory tract (e.g., filamentous hemagglutinin and periactin) and molecules that cause local and systemic damage of the host (pertussis toxin, or PT)...PT plays a central role in the pathogenesis of whooping cough and induces protective immunity against infection. As for the other toxins, to be included in vaccines, PT needs to be detoxified. In the early 1980s, during the development of acellular pertussis vaccines, a number of chemical methods (formaldehyde, hydrogen peroxide, tetranitromethane, and gluteraldehyde) were used to detoxify PT. Although many of the vaccines in use today
contain a formaldehyde detoxified PT, genetic engineering was used to detoxify PT.” From: Bacterial Protein Toxins, Edited by Drusilla Butnsd et al, ASM Press, 2003.

- PT is a protein of 105,000 daltons composed of five noncovalently linked subunits named S1 through S5, and organized into two functional domains called A and B. The A domain, which is composed of the S1 subunit, is an enzyme that intoxicated eukaryotic cells by ADP-ribosylating their GTP-binding proteins…The B domain is a nontoxic oligomer…Many mutants containing single or multiple amino acid substitutions in the A or B subunits have been constructed by site directed mutagenesis of the PT gene…The most popular one is PT-9K/129G…The nontoxic mutant is being used for vaccination against pertussis and therefore is produced in gram quantities. From: Guidebook to Protein Toxins and Their Use in Cell Biology, Edited by Rino Rappuoli, Sambrook & Tooze Publication at Oxford University Press, 1997.

- “Pertussis toxin was discovered in the late 1960s and originally named islet-activating protein or lymphoctytosis-promoting factor. It was purified soon thereafter. It also has a heterohexameric structure, but is composed of five different subunits, S1 through S5, with two molecules of S4 per holotoxin molecule. The S1 subunit has ADP-RT activity, while the receptor-binding oligomer is formed from dimmers of S2S4 and S3S4 subunits, linked by the SF subunit. The locus encoding PT was cloned in 1986 and shown to encode a five gene operon. Each gene in the operon encodes a subunit with a predicted signal sequence that directs the mature polypeptide to periplasmic space. Predicted molecular masses of the mature polypeptides agrees with their sizes as seen by SDS-Page of purified holotoxin….The contribution of PT to the symptoms of whooping cough is more difficult to elucidate since B. pertussis has multiple virulence factors and toxins, including tracheal cytotoxin, dermonecrotic toxin and a bifunctional anenyulate cyclase-hemolysin. …Modulation of the immune system by PT is responsible for some of the symptoms of the disease such as lymphocytosis, and PT has been used experimentally as an adjuvant to induce organ specific immune diseases.” From Microbial Toxins: Molecular and Cellular Biology, edited by Thomas Proft, Horixon Bioscience, 2005.


- Diagnostic Tests/Clinical Signs & Symptoms
Diagnostic testing is not useful in acute management, but blood serum should be obtained and held for later analysis. Clinical signs and symptoms should guide clinical treatment.

- Pre-exposure Prophylaxis
A vaccine containing toxoid or a genetically inactivated toxin is available, and all potentially exposed employees must be fully immunized and may need to receive a booster dose every ten years (to be determined).

- Post-exposure Prophylaxis/Treatment:
Pertussis immune globulin may be helpful, if available. Pertussis immune serum has been in use since the 1940s.

II. ADDITIONAL BACKGROUND INFORMATION
DESCRIPTION AND IMPLICATIONS OF RISK:

- The primary pertussis vaccine series is usually given in a combination injection with tetanus and diphtheria vaccines, and is known as the DTP vaccine. A child should have received four DTP shots by 18 months of age, with a booster shot given between the ages of 4 to 6 years. After that, pertussis, diphtheria and tetanus boosters (Tdap) should be given once as an adult. However, the recommendation for one time immunization only may change to reimmunization every 10 years to provide continued protection.
AGENT SUMMARY STATEMENT
Agent: Bordetella pertussis

LABORATORY SAFETY AND CONTAINMENT RECOMMENDATIONS
The agent may be present in high levels in respiratory secretions, and may be found in other clinical material, such as blood and lung tissue in its infrequent manifestation of septicemia and pneumonia, respectively. Because the natural mode of transmission is via the respiratory route, aerosol generation during the manipulation of cultures and contaminated clinical specimens generates the greatest potential hazard.
BSL-2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of known or potentially infectious clinical material and cultures. ABSL-2 practices and containment equipment should be employed for housing experimentally infected animals. Primary containment devices and equipment, including biological safety cabinets, safety centrifuge cups or safety centrifuges should be used for activities likely to generate potentially infectious aerosols. BSL-3 practices, containment equipment, and facilities are appropriate for production operations.

SPECIAL ISSUES

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country.

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. 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. 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. The main laboratory risks 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.

TRAINING AND LABORATORY PLANNING
Each laboratory worker must be trained in the theory and practice of the toxins to be used, with special emphasis on the nature of the practical hazards associated with laboratory operations. This includes how to handle transfers of liquids containing toxin, where to place waste solutions and contaminated materials or equipment, and how to decontaminate work areas after routine operations, as well as after accidental spills. The worker must be reliable and sufficiently adept at all required manipulations before being provided with toxin. 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, animal safety practices must be considered for toxin work involving animals.

Each laboratory that uses toxins should develop a specific chemical hygiene plan. The National Research Council has provided a review of prudent laboratory practices 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 handling laboratory equipment used to manipulate the toxin or organism.

SAFETY EQUIPMENT AND CONTAINMENT
Routine operations with dilute toxin solutions are conducted under BSL-2 conditions with the aid of personal protective equipment and a well-maintained BSC or comparable engineering controls. 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. 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.

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

INADVERTENT 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 is taken from the centrifuge to a BSC to open it and remove its tubes.

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 decontaminated as soon as is practical. Glassware should be replaced with plastic 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 respiratory protection and engineering controls. 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. Aerosol exposure of animals should be done in a certified Class III BSC or hoodline. While removing exposed animals from the hoodline and for required animal handling during the first 24 h after exposure, workers should take additional precautions, including wearing protective clothing (e.g., disposable Tyvek suit) and appropriate respiratory protection. To minimize the risk of dry toxin generating a secondary aerosol, areas of animal skin or fur exposed to aerosols should be gently wiped with a damp cloth containing water or buffered cleaning solution before the animals are returned to holding areas. 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, and some protein toxins possess a capacity to re-fold, and partially reverse inactivation caused by heating. In addition, the conditions for denaturing toxins in aqueous solutions are not necessarily applicable for inactivating dry, powdered toxin preparations. 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, autoclaved and disposed of as toxic waste. 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 toxic 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. Decontamination of buildings or offices containing sensitive equipment or documents poses special challenges. Large-scale decontamination is not covered explicitly here, but careful extrapolation from the basic principles may inform more extensive clean-up efforts.


ADDITIONAL REFERENCES


California Aerosol Transmissible Disease Standard:  http://www.dir.ca.gov/Title8/5199.html