Organism or Agent: Pertussis Toxin
Exposure Risk: Multiple Endocrine/Metabolic Effects
Exposure Hotline Pager: 415/353-7842 (353-STIC) (Available 24 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 Biosafety Officer 415/514-2824
EH&S Public Health Officer: 415/514-3531
UCSF Occupational Health Services: 415/885-7580 (Available during work hours)
California Poison Control: 800/222-1222
SFDPH Emergency Number: 415/554-2830
CDC Emergency Operations: 770/488-7100

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. If working with pertussis, decontaminate any exposed skin 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 inform their supervisor, and take a copy of this entire protocol document to the Emergency Department, including information regarding the specific toxin subtypes associated with exposure.

4. Follow-up is needed in the event of any Laboratory Exposure:
   a. 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.
1. **WORKER’S RESPONSIBILITIES** (Employee/Student Initial Self-Care)
   a. **First Aid:** Perform recommended first aid and decontamination according to the posted instructions. Decontaminate any exposed skin surfaces.
   b. **Treatment:** i) In the event of an acute exposure or injury resulting from a laboratory, the injured individual should report to the Emergency Department for acute medical treatment. The employee should bring a copy of this protocol. The employee should 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 Exposure Hotline in order to get access to medical care for the exposure and evaluation for possible post exposure prophylaxis.
   c. **Access to the Exposure Hotline:** Immediately call the Exposure Hotline in the event of an exposure. Dial 415/353-7842 and provide your name and contact information to the operator. If there is no call back in 15 minutes, call again. If there is no call back the second time, proceed to the nearest Emergency Department 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://ucsfhr.ucsf.edu/files/EIR.pdf](http://ucsfhr.ucsf.edu/files/EIR.pdf)
   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. Remove any garments that may have become soiled/contaminated and place them in a double plastic bag.
   g. **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 Environment, 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 Biosafety 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

The exposure incident should also be reported to the San Francisco Department of Public Health as required by the Cal/OSHA ATD standard.

e. **Follow-Up:** Confirm that the worker has called for an appointment at the UCSF Occupational Health Clinic.


### 3. PRINCIPAL INVESTIGATOR 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 Bordatella 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). The PI shall ensure that any known exposure is reported to the Biosafety officer and to the San Francisco Department of Public Health.

### 4. EMERGENCY DEPARTMENT RESPONSIBILITIES

a. Onset of symptoms following significant toxin exposure would typically be delayed by days to weeks. The Emergency Department shall assess the severity of the exposure, and take appropriate actions to include consultation with the California Poison Control System.

b. The emergency room should draw at least ten milliliters of serum and hold it for possible toxin assay. This must be done before any treatment with antitoxin.

c. Any patient seen in the Emergency Department and released should be given information about the potential for delayed onset of symptoms/toxicity. Any symptoms would be reason for emergent reevaluation. Any exposed individuals should also be referred to the UCSF Occupational Health Services for follow-up care.
d. The **Emergency Department** and UCSF Occupational Health Services both need to complete a Doctor’s First Report of Occupational Illness (DFR). The Emergency Department physician should leave a message for Occupational Health that an exposure has occurred. The physician administering care should forward a copy of the DFR to UCSF HR. Here is a link to the form: [http://www.dir.ca.gov/dlsr/dlsrform5021.pdf](http://www.dir.ca.gov/dlsr/dlsrform5021.pdf)
SAFETY DATA SHEET – Pertussis Toxin

NAME: Pertussis toxin from Bordetella pertussis

I. HAZARDS IDENTIFICATION

Emergency Overview
OSHA Hazards
Target Organ Effect, Highly toxic by inhalation, Toxic by ingestion, Toxic by skin absorption
Target Organs
Pancreas.
HMIS Classification
Health Hazard: 4
Chronic Health Hazard: *
Flammability: 0
Physical hazards: 0
NFPA Rating
Health Hazard: 4
Fire: 0
Reactivity Hazard: 0
Potential Health Effects
Inhalation May be fatal if inhaled. May cause respiratory tract irritation.
Skin Toxic if absorbed through skin. May cause skin irritation.
Eyes May cause eye irritation.
Ingestion Toxic if swallowed.

II. FIRST AID MEASURES

General advice
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.

III. FIRE-FIGHTING MEASURES

Flammable properties
Flash point no data available
Ignition temperature no data available
Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Special protective equipment for fire-fighters
Wear self-contained breathing apparatus for fire fighting if necessary.

IV. ACCIDENTAL RELEASE MEASURES

Personal precautions
Wear respiratory protection. Avoid dust formation. Avoid breathing dust. Ensure adequate ventilation. Evacuate personnel to safe areas.

Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

Methods for cleaning up
Pick up and arrange disposal without creating dust. Keep in suitable, closed containers for disposal.

V. HANDLING AND STORAGE

Handling
Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.

Storage
Keep container tightly closed in a dry and well-ventilated place. Recommended storage temperature: 2 - 8 °C

VI. EXPOSURE CONTROLS/PERSONAL PROTECTION

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.

Hygiene measures
Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

VII. PHYSICAL AND CHEMICAL PROPERTIES

Appearance
Form solid

Safety data
pH no data available
Melting point no data available
Boiling point no data available
Flash point no data available
Ignition temperature no data available
Lower explosion limit no data available
Upper explosion limit no data available
Water solubility no data available

VIII. STABILITY AND REACTIVITY

Storage stability
Stable under recommended storage conditions.

Materials to avoid
Strong oxidizing agents

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. Nature of decomposition products not known.

IX. TOXICOLOGICAL INFORMATION

Acute toxicity
LD50 Intravenous - rat - 0.114 mg/kg
Remarks: Sense Organs and Special Senses (Nose, Eye, Ear, and Taste)
Eye: Lacrimation.
Behavioral: Change in motor activity (specific assay). Nutritional and Gross
Metabolic: Weight loss or decreased weight gain.

Irritation and corrosion
no data available

Sensitisation
no data available

Chronic exposure
IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.
ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.
NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.
OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

Potential Health Effects

Inhalation May be fatal if inhaled. May cause respiratory tract irritation.

Skin Toxic if absorbed through skin. May cause skin irritation.

Eyes May cause eye irritation.

Ingestion Toxic if swallowed.

Target Organs Pancreas,

Additional Information
RTECS: XW5883750

X. ECOLOGICAL INFORMATION

Elimination information (persistence and degradability)
no data available

Ecotoxicity effects
no data available

Further information on ecology
no data available

XI. DISPOSAL CONSIDERATIONS

Product
Observe all federal, state, and local environmental regulations. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

**Contaminated packaging**
Dispose of as unused product.

XII. TRANSPORT INFORMATION

**DOT (US)**
Sigma - P140
Sigma-Aldrich Corporation
www.sigma-aldrich.com Page 5 of 6
UN-Number: 3462 Class: 6.1 Packing group: I
Proper shipping name: Toxins, extracted from living sources, solid, n.o.s. (Pertussis toxin from Bordetella pertussis)
Marine pollutant: No

**IMDG**
UN-Number: 3462 Class: 6.1 Packing group: I
Proper shipping name: TOXINS, EXTRACTED FROM LIVING SOURCES, SOLID, N.O.S. (Pertussis toxin from Bordetella pertussis)
Marine pollutant: Marine pollutant

**IATA**
UN-Number: 3462 Class: 6.1 Packing group: I
Proper shipping name: Toxins, extracted from living sources, solid n.o.s. (Pertussis toxin from Bordetella pertussis)

XIII. REGULATORY INFORMATION

**OSHA Hazards**
Target Organ Effect, Highly toxic by inhalation, Toxic by ingestion, Toxic by skin absorption

**DSL Status**
This product contains the following components that are not on the Canadian DSL nor NDSL lists.
Pertussis toxin from Bordetella pertussis
CAS-No.
70323-44-3

**SARA 302 Components**
SARA 302: No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

**SARA 313 Components**
SARA 313: This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

**SARA 311/312 Hazards**
Acute Health Hazard, Chronic Health Hazard

**California Prop. 65 Components**
This product does not contain any chemicals known to State of California to cause cancer, birth, or any other reproductive defects.

Taken from:
ADDITIONAL REFERENCES


CDC website on vaccine preventable diseases:  http://www.cdc.gov/vaccines/pubs/pinkbook/pert.html

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

SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

Bordetella pertussis - Safety Data Sheets (SDS)

SAFETY DATA SHEET - INFECTIOUS SUBSTANCES
SECTION I - INFECTIOUS AGENT

NAME: Bordetella pertussis

SYNONYM OR CROSS REFERENCE: Whooping cough, B. parapertussis

CHARACTERISTICS: Gram-negative coccobacilli, aerobic, encapsulated, non-motile, produces a toxin; B. pertussis has fastidious growth requirements while B. parapertussis grows on simple culture media

SECTION II - HEALTH HAZARD

PATHOGENICITY: 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: 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

INFECTIONDOSE: Unknown

MODE OF TRANSMISSION: Primarily by direct contact with discharges from respiratory mucous membranes of infected persons by the airborne route, probably by droplets; frequently brought home by an older sibling

INCUBATION PERIOD: Commonly 6 to 20 days; average 7 to 10 days

COMMUNICABILITY: Highly communicable in the early catarrhal stage; becomes negligible in about 3 weeks despite persisting spasmodic cough with whoop; when treated with antibiotics, the period of infectiousness extends only 5-7 days after onset of therapy
SECTION III - DISSEMINATION

RESERVOIR: Infected persons; asymptomatic carriers (both children and adults)

ZOONOSIS: None

VECTORS: None

SECTION IV - VIABILITY

DRUG SUSCEPTIBILITY: Sensitive to erythromycin or TMP-SMX (susceptible to ciprofloxacin in vitro)

DRUG RESISTANCE: Two erythromycin-resistant isolates of *B. pertussis* have been reported in Utah and Arizona

SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to many disinfectants - 1% sodium hypochlorite, 70% ethanol, iodines, phenolics, glutaraldehyde, formaldehyde

PHYSICAL INACTIVATION: Inactivated by moist heat (121°C for at least 15 min) and dry heat (160-170°C for at least 1 hour)

SURVIVAL OUTSIDE HOST: Survives 1-2 hours on surfaces; 3-4 hours in human sputum samples; air - 19 to 20 hours; plastic - 3-5 days; paper - 1 day; in diluted saliva - up to 7 days; susceptible to cold temperatures and dessication

SECTION V - MEDICAL

SURVEILLANCE: Monitor for symptoms; confirmation by recovery of organism from nasopharyngeal swabs during catarrhal stage

FIRST AID/TREATMENT: Antibiotic therapy - 14 day course of erythromycin or TMP-SMX; adequate oxygenation, hydration and electrolyte balance

IMMUNIZATION: Whole cell adsorbed vaccine available; recommended for children at 2 months of age, boosted at 2 and 5 years; acellular engineered fraction vaccines licensed for booster vaccination

PROPHYLAXIS: Close contacts less than 7 years who have not received required DTP doses should be given a DTP dose; 18-day erythromycin or TMP-SMX course for close contacts less than 1 year old, regardless of immunization status, and for unimmunized contacts less than 7 years

SECTION VI - 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

SPECIAL HAZARDS: No special hazards

SECTION VII - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices, containment equipment and facilities for work with known or potentially infectious specimens and cultures; work likely to generate aerosols should be carried out in a biosafety cabinet

PROTECTIVE CLOTHING: Laboratory coat; gloves when direct contact with infectious materials is unavoidable; gloves and gown (tie in back, tight wrists) should be worn while conducting procedures in biosafety cabinet

OTHER PRECAUTIONS: Hands should be washed thoroughly after work is finished to avoid possible spreading of the organisms

SECTION VIII - HANDLING INFORMATION

SPILLS: Allow aerosols to settle; wearing protective clothing, gently cover spill with paper towel and apply 1% sodium hypochlorite, starting at perimeter and working towards the centre; allow sufficient contact time (30 min) before clean up

DISPOSAL: Decontaminate before disposal; steam sterilization, chemical disinfection, incineration

STORAGE: In sealed containers that are labelled appropriately

SECTION IX - MISCELLANEOUS INFORMATION

Date prepared: November 1999

Prepared by: Office of Laboratory Security, PHAC

Although the information, opinions and recommendations contained in this Material Safety Data Sheet are compiled from sources believed to be reliable, we accept no responsibility for the accuracy, sufficiency, or reliability or for any loss or injury resulting from the use of the information. Newly discovered hazards are frequent and this information may not be completely up to date.

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](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 restrained 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 peractin) 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 Butler 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 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 lymphocytosis-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.

Overview (Portions taken directly from BMBL: http://www.cdc.gov/biosafety/publications/bmbl5/index.htm)

- **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.
• Post-exposure Prophylaxis/Treatment:
  Pertussis immune globulin may be helpful, if available. Pertussis immune serum has been in used 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 as an adult every 10 years to provide continued protection.

• Studies show that as age increases, the circulating antibody levels progressively decline.
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
Vaccines The reader is advised to review the current recommendations of the ACIP published in the Morbidity and Mortality Weekly Report (MMWR) and at the CDC Vaccines and Immunizations Web site for the latest recommendations for adolescents and adults.

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 toxics 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 toxics. 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 denaturizing 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.

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.