

Safety Data Sheet

Pertussis Toxin

Division of Safety
National Institutes
of Health



WARNING!

THIS GROUP OF COMPOUNDS IS ACUTELY TOXIC. THEY ARE READILY ABSORBED THROUGH THE RESPIRATORY TRACT. THEY MAY IRRITATE TISSUES (SKIN, EYES, MUCOUS MEMBRANES, AND LUNGS) AND INDUCE SENSITIVITY. AVOID FORMATION AND BREATHING OF AEROSOLS.

LABORATORY OPERATIONS SHOULD BE CONDUCTED IN A FUME HOOD, GLOVE BOX, OR VENTILATED CABINET.

AVOID SKIN CONTACT: IF EXPOSED, WASH WITH SOAP AND COLD WATER. AVOID WASHING WITH SOLVENTS. AVOID RUBBING OF SKIN OR INCREASING ITS TEMPERATURE.

FOR EYE EXPOSURE, IRRIGATE IMMEDIATELY WITH LARGE AMOUNTS OF WATER. FOR INGESTION, DRINK MILK OR WATER. REFER FOR GASTRIC LAVAGE. FOR INHALATION, REMOVE VICTIM PROMPTLY TO CLEAN AIR. ADMINISTER RESCUE BREATHING IF NECESSARY. REFER TO PHYSICIAN.

IN CASE OF LABORATORY SPILL, WEAR PROTECTIVE CLOTHING DURING CLEANUP. AVOID SKIN CONTACT OR BREATHING OF AEROSOLS. DISPOSE OF WASTE SOLUTIONS AND MATERIALS APPROPRIATELY.

A. Background

The aerobic bacterium Bordetella pertussis produces three major toxins (or classes of toxins), all of which appear to be involved in the etiology of the disease pertussis (whooping cough) in humans. They are:

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1. Heat-labile or dermonecrotizing toxin (HLT);^A
2. Lipopolysaccharide (LPS) endotoxin; and,
3. A protein, variously named:
 - a. histamine sensitization factor (HSF),
 - b. lymphocytosis (or leukocytosis) - promoting factor (LPF)
 - c. islet activating protein (IAP),
 - d. pertussis toxin, (PTx),
 - e. pertussigen.

While many American investigators believe that the functions described under this last item are attributable to one and the same protein, this view is not shared by other, particularly Japanese, authors who maintain that there is considerable evidence against this "unitarian hypothesis" and use the individual names in their publications.^B A major difficulty in deciding for or against this hypothesis arises from the fact that man is the only species subject to natural infection with pertussis, and the only one exhibiting the paroxysmal cough syndrome typical of whooping cough. For this reason also the molecular basis of toxic activity is not well understood, and there is potential for interaction between the three classes of compounds as well as other products of B. pertussis.

Recent reviews include Wardlaw and Parton (1983) and Wilfert (1984)

B. Chemical and Physical Data

1. Chemical Abstract Nos.: None.
2. Synonyms: Listed in A above.
3. Chemical structures and molecular weights:
 - a. HLT: Most investigations have employed only partially purified HLT; one publication (Nakase et al., 1969) describes HLT in purified form (free from hemagglutinins, antigens, and agglutinogens) as a glycoprotein containing no phosphorus or lipids. The carbohydrate component contains mannose and an unknown material. An apparent

^AThe abbreviations used here will be used throughout the body of this Safety Data Sheet.

^BThe use of the term "PTx" usually implies the gamut of activities enumerated in paragraph 3 above.

molecular weight of 89,000 has been listed but the authors (Wardlaw and Parton, 1983) believe that this may be due to a combination of HLT with other proteins.

b. LPS: This component is made of two liposaccharides which consist of two polysaccharides (PSI, PSII) and two lipids (Lipid A, Lipid X). Lipid A is an esterified phosphate containing tetradecanoic, 3-hydroxydecanoic, and 3-hydroxy-tetradecanoic acid. Lipid X, a minor component, contains the above plus 2-methyl-3-hydroxydecanoic and 2-methyl-2-hydroxytetradecanoic acid. PSI and PSII are bound to Lipid A through one molecule of KDO (3-deoxy-2-octulosonic acid) and phosphorylated KDO, respectively. Molecular weights of PSI and PSII are 2,800 and 3,600, respectively (Wardlaw and Parton, 1983).

c. PTx: In two publications this has been described in two ways:

(1) Composed of four polypeptide chains, dissociated from each other by 8 M urea; two of these chains are identical, so that there are three types of chains in proportion $F_1:F_2:F_3 = 1:2:1$. Molecular weights are listed as 44, 20, and 11×10^3 for the three types (Wilfert, 1984).

(2) Composed of five polypeptide chains (S_1-S_5) with molecular weights 26.3, 24.4, 22.7, 12.2 and 11.3×10^3 , respectively. S_4 is calculated to contain one cysteine moiety and the other four at least two each, possibly with intrachain disulfide bonds (Peppler et al., 1985).

There is obviously still an uncertainty about the true composition of PTx.

Density: No data on any component.

Absorption spectroscopy: No data on any component.

Volatility: May be considered negligible.

Solubility: Crystalline PTx is soluble in 0.1 M sodium pyrophosphate, pH 10.5, which is adjusted with Tris buffer for biological experiments (Arai and Munoz, 1981). No data on other components. Pertussis vaccine is soluble in dilute saline solution.

Description: Crystalline PTx consists of fine rods. For other descriptions see B3 above.

9. Boiling point, melting point: Not applicable.

10. Stability:

- a. HTL loses all activity at 56°C in 10 minutes (Wardlaw and Parton, 1983). Crude preparations are stable up to two weeks at 4°C and show 10% loss of toxicity in one year at -20°C (Livey and Wardlaw, 1984).
- b. LPS: Stated to be "heat-stable" (Wilfert, 1984), without quantitative data; this appears reasonable for a lipopolysaccharide.
- c. PTx in solution maintains full activity at 4°C for 24 hours at pH 4-10 but is inactivated outside of this pH range. At pH 7, it is stable for 15 min at 50°C but is inactivated at 60°C and above (Wardlaw and Parton, 1983).

11. Chemical reactivity:

- a. HLT is inactivated by formaldehyde, phenol, chloroform, ethanol, and trypsin.
- b. LPS: No data; the polysaccharide components of LPS are probably subject to reactions with the usual carbohydrate reagents.
- c. PTx is detoxified by reaction with glutaraldehyde; detoxified PTx can protect mice against intracerebral challenges with B. pertussis cells (Munoz et al., 1981). No other data, but PTx presumably reacts with common protein reagents.

12. Flash point: Not applicable.

13. Autoignition temperature: Not applicable.

14. Explosive limits in air: Not applicable.

Fire, Explosion, and Reactivity Hazard Data

1. Pertussis toxins do not require special fire-fighting procedure or equipment and do not present unusual fire and explosion hazards.
2. No incompatibilities are known.
3. Pertussis toxins do not require non-spark equipment.

Operational Procedures

It should be emphasized that this data sheet and the NIH Guidelines are intended as starting points for the implementation of good

laboratory practices when using this compound. The practices and procedures described in the following sections pertain to the National Institutes of Health and may not be universally applicable to other institutions. Administrators and/or researchers at other institutions should modify the following items as needed to reflect their individual management system and current occupational and environmental regulations.

1. Chemical inactivation/decontamination: See B11, above.
2. Disposal: It may be possible to decontaminate waste streams containing pertussis toxins before disposal. For details, see B 11, above. No waste streams containing pertussis toxins shall be disposed of in sinks or general refuse. Chemical waste streams contaminated with pertussis toxins shall be handled as hazardous chemical waste and disposed of in accordance with the NIH chemical waste disposal system. Nonchemical waste (e.g., animal carcasses and bedding) containing pertussis toxins shall be handled and packaged for incineration in accordance with the NIH medical-pathological waste disposal system. Potentially infectious waste (e.g., tissue cultures) containing pertussis toxins shall be disinfected by heat using a standard autoclave treatment and packaged for incineration, as above. Burnable waste (e.g., absorbent bench top liners) minimally contaminated with pertussis toxins shall be handled as potentially infectious waste and packaged for incineration, as above. Absorbent materials (e.g., associated with spill cleanup) grossly contaminated shall be handled in accordance with the chemical waste disposal system. Radioactive waste containing pertussis toxins shall be handled in accordance with the NIH radioactive waste disposal system.
3. Storage: Store solid pertussis toxins and their solutions in dark-colored, tightly closed containers in a deep freeze. Avoid exposure to light and moisture.

Monitoring and Measurement Procedures Including Direct Field Measurements and Sampling for Subsequent Laboratory Analysis

1. Sampling: No data.
2. Analysis:
 - a. HLT: A method based on size of pale spots produced by intracutaneous inoculation of rabbits has been described (Kurokawa et al., 1969).
 - b. LPS: No published procedures.
 - c. PTx: A specific growth effect on Chinese hamster ovary cells in tissue culture has been made the basis of an in

vitro assay, with a minimum detectable dose of less than 1 ng/ml (Hewlett et al., 1983); it should be noted, however, that this method was developed with purified but not with crystalline PTx, so there may be some doubt as to specificity. Another method (seen in abstract form only) is an enzyme-linked immunoabsorbent assay, based on specific affinity of PTx for human haptoglobulin (Sato et al., 1983).

Biological Effects (Animal and Human)

Introductory note: This document is not considered to be the place for a discussion of the etiology of pertussis, for which standard medical texts should be consulted; it is also reviewed by Wilfert (1984). The relationship between the various biological effects described below and the disease is difficult to evaluate since man is the only species susceptible to infection with B. pertussis and he exhibits the symptoms typical for this disease.

1. Absorption: No specific data. The individual components (HLT, LPS, PTx) produce biological effects following parenteral injection, and the neurotoxic action of HLT is exhibited after intracutaneous inoculation. The respiratory route is considered to be the main entry of the infective organism.
2. Distribution and pharmacokinetics: No data.
3. Metabolism and excretion: No data. Presumably the metabolic pathway of proteinaceous material (HLT, PTx) is that of usual proteolysis, and that of LPS is hydrolysis to lipid and polysaccharide and further catabolism. No studies with isotopically labeled toxin fractions appear to have been carried out.
4. Toxic effects: There are few acute toxicity estimations. For HLT an LD50 in mice of 16.5 $\mu\text{g}/\text{kg}$ and a minimal necrotizing dose in guinea pigs of 0.01 μg (Wardlaw and Parton, 1983), and for PTx an intraperitoneal LD50 in mice of 429 or 546 ng per mouse (Arai and Munoz, 1981; Munoz et al., 1981) have been reported.

HLT is dermonecrotic in mice, guinea pigs, and rabbits. Intravenous injection in mice and guinea pigs results in an increased leukocyte count, decreased spleen weight and necrosis of liver, spleen, and kidney (Nakase et al., 1969). Its role in human disease is unknown. LPS is a typical endotoxin: it is toxic (no quantitative data), pyrogenic, and Schwartzman reactive (Wardlaw and Parton, 1983).

The toxic functions of PTx are best described by the various names given to this toxin (see A above) and have been reviewed (Wilfert, 1984). It promotes lymphocytosis, induces histamine sensitization, and hypoglycemia by stimulating insulin secretion. This latter (IAP) effect is caused by binding of the

toxin to receptor sites on pancreatic islets, insertion through the cell membrane resulting in calcium influx, stimulation of the adenylate cyclase system, and consequent insulin release. This effect has been demonstrated in vitro (Katada and Ui, 1982).

A model which perhaps most closely resembles the human response to pertussis toxins consists of cultures of proliferating epithelial cells derived from hamster trachea; these cells are able to bind virulent B. pertussis cells. In this system there is inhibition of DNA synthesis, decreased ciliary activity, and eventual extrusion of ciliary cells; these effects are produced both by supernatants of B. pertussis broth cultures and, upon purification, by "tracheal cytotoxin" (TCT), of molecular weight 1,500, which consists of amino acids and amino sugars (Goldman et al., 1982). Further work with TCT, particularly a study of whether it produces in vivo the other effects ascribed to PTx, should lead to resolution of arguments for and against the "unitarian hypothesis" of PTx toxic action.

5. Carcinogenic effects: None have been reported.
6. Mutagenic and teratogenic effects: No data.

Emergency Treatment

1. Skin and eye exposure: For skin exposure, remove contaminated clothing and wash skin with soap and water. Skin should not be rinsed with organic solvents. Avoid rubbing of skin or increasing its temperature. For eye exposure, irrigate immediately with copious quantities of running water for at least 15 minutes. Obtain ophthalmological evaluation.
2. Ingestion: Drink plenty of water or milk. Vomiting might reexpose the mouth and esophagus. Refer for gastric lavage.
3. Inhalation: Remove victim promptly to clean air. Administer rescue breathing if necessary.
4. Refer to physician at once. Consider treatment for pulmonary irritation.

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