

Safety Data Sheet

Botulinum Toxin

Division of Safety
National Institutes
of Health



WARNING!

THIS GROUP OF COMPOUNDS IS HIGHLY TOXIC. THEY ARE READILY ABSORBED THROUGH THE INTESTINAL TRACT AND TRANSPLACENTALLY. THEY MAY 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 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

There have been identified to date eight types of botulinum toxins (BTs) which have been designated as type A, B, C₁, C₂, D, E, F, and G. They are produced by different strains of Clostridium botulinum which partially overlap in their production of different types. The designation of these strains, with some of their characteristics, are as follows:

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<u>Strain Group</u>	<u>BT type produced</u>	<u>Characteristics</u>			<u>Heat stability of spores</u>
		<u>proteolytic</u>	<u>saccharolytic</u>	<u>gelatinolytic</u>	
I	all A, some B, F	+	-	-	high
II	all E, some B, F	-	+		low
III	all C and D	-	+	+	intermediate
IV	all G	+	-		

BTs are the most toxic (natural or synthetic) materials known; for instance, the LD₅₀ of type A has been estimated to be 0.025 ng or less than 10⁸ molecules of toxin per mouse (Bonventre, 1979). All appear to be simple proteins. Type E differs from the others in that it is produced by the organism in nontoxic form which is activated by trypsin. BTs are neurotoxins whose lethality is produced through paralysis of skeletal, particularly the respiratory, muscles. Botulism in man is usually food-borne, most commonly associated with types A, B, and E, or due to wound infection by spores. There is evidence for types C₁ and D that toxin production is governed by viral infection of the organism, and this may also be true for other types.

A historical review (literature prior to 1959) has been presented by LaManna (1959). More recent reviews include Simpson (1981), Das Gupta (1983), Sakaguchi (1983), Sakaguchi et al. (1984), Hill et al. (1984), Habermann and Dreyer (1986).

Chemical and Physical Data

1. Chemical Abstract Nos.: None.
2. Synonyms: None.
3. Chemical structures and molecular weights: The botulin neurotoxins are proteins of molecular weights ca. 150,000 (with variations between 141,000 and 167,000), usually associated with nontoxic components of m.w. 150,000 or higher. For instance, crystalline BT type A (m.w. 900,000) consists of one molecule of neurotoxin (m.w. 150,000) and four molecules of hemagglutinin which dissociate reversibly at slightly alkaline pH and irreversibly at pH 9.2 or higher. The m.w. 150,000 neurotoxins are in turn composed of two chains of m.w. ca. 100,000 and ca. 50,000 respectively, which are held together by one or more disulfide bridges. The various molecular sizes are often classified by their sedimentation constants as 7S, 12S, 16S, and 19S, or as S (small), M (medium), L (large), or LL (extra large). The 50,000 size (S or 7S) represents the true neurotoxic unit. For a detailed discussion see Sakaguchi (1983).

As a general rule, the larger the molecular size of a given toxin, the higher the oral toxicity, and the lower the parenteral toxicity and stability. It has been inferred from this that the lower m.w. active neurotoxin is protected from inactivation by gastrointestinal juices by its combination with higher m.w., nontoxic components. See for instance the pertinent discussion on this subject for BT type E (Kitamura et al., 1969a, b). The "nicking" of BTs by trypsin will not be discussed since it seems to have no effect on toxicity (Ohishi and Sakaguchi, 1977).

The above general description applies to all BTs except type C₂. This toxin (which also differs from the others in having toxic actions at sites other than the nervous system) is composed of two covalently linked chains of m.w. 100,000 and 50,000, both of which are required for toxic activity but need not be administered simultaneously; BT type C₂ has therefore been called a "binary toxin" (Simpson, 1982; Simpson, 1984).

The existence of highly toxic BTs of much lower m.w. (12,000-18,000), derived from preparations of type A, B, and E toxins, was postulated by Gerwing et al. (1964, 1965). The findings of this group could not be duplicated in other laboratories and have been criticized on procedural grounds (e.g., Knox et al., 1970a).

Amino acid compositions have been determined for most types of BTs (e.g., Sathyamoorthy and Das Gupta, 1985; Schmidt et al., 1985; Das Gupta and Rasmussen, 1983). It has been noted (for type B at least) that close to half of the amino acids are of the hydrophobic type (Beers and Reich, 1969).

Density: No data on any type.

Absorption spectroscopy: Very few data, but in general one may assume typical absorption characteristics of proteins. BT type B has an ultraviolet absorption maximum at 278 nm and shows fluorescence ($\lambda_{ex} = 287$ nm, $\lambda_{em} = 323$ nm) (Beers and Reich, 1969).

Volatility: May be considered negligible.

Solubility: BTs are usually administered in saline solution. No quantitative data.

Description: BT type A is the only one which has been crystallized. For other descriptions see B3 above.

Isoelectric point: 6.1 for A, 5.25 for B, 5.7 for F (Das Gupta, 1983).

Boiling point, melting point: Not applicable.

1. **Stability:** As noted above, stability of BTs decreases with purification and separation from nontoxic adjuncts to the neurotoxins. Solutions of crystalline type A at concentrations of 2 mg/ml in acetate, pH 4.2 are stable for long periods of time, but lower concentrations deteriorate rapidly unless stabilized by addition of gelatin and bovine serum albumin: with these additions, solutions of 100 ng/ml are stable for two years at room temperature. Toxicity of unstabilized solutions is destroyed by freezing (Schantz and Scott, 1981). Type B toxin (m.w. 167,000, i.e., the dichain described in B3) in phosphate buffer, pH 5.7, is stable for at least 8 months when stored at 4°C (Beers and Reich, 1969). All BT solutions are destroyed by boiling for one minute, or at 75-80°C for 5-10 minutes. Exposure to light destroys BTs rapidly, more slowly when protected from atmospheric oxygen (Hill et al., 1984).
2. **Chemical reactivity:** Toxicity of BTs is associated with intactness of
 - a. **tryptophan residues:** photo-oxidation of type A in presence of methylene blue results in loss of 17 moles of tryptophan per mole of BT and 99.4% of toxicity (Boroff et al., 1967); and
 - b. **disulfide bridges:** reduction with dithioerythritol (Sugiyama et al., 1973) and other reducing agents (Simpson, 1981) results in loss of neurotoxicity. BTs react with typical sulfhydryl reagents (p-chloromercuribenzoate, iodoacetic acid, iodoacetamide) but this reaction is slow and partial, and it is believed that sulfhydryl groups are not an integral part of their active centers (Knox et al., 1970b). Treatment with formaldehyde is the usual procedure for the production of toxoid.

BTs are readily inactivated by alkali (e.g., 0.1 N sodium hydroxide).

- . **Flash point:** Not applicable.
- . **Autoignition temperature:** Not applicable.
- . **Explosive limits in air:** Not applicable.

Fire, Explosion and Reactivity Hazard Data

- . BTs do not require special fire-fighting procedures or equipment and do not present unusual fire and explosion hazards.
- . No incompatibilities are known.
- . BTs do not require nonspark 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 these compounds. 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 B12 above.
2. Decontamination: Turn off equipment that could be affected by BTs or the materials used for cleanup. If there is any uncertainty regarding the procedures to be followed for decontamination, call the NIH Fire Department (dial 116) for assistance. Wipe off surfaces with 0.1 N NaOH, then wash with copious quantities of water. Glassware should be rinsed (in a hood) with 0.1 N NaOH, followed by soap and water. Animal cages should be washed with water.
3. Disposal: It may be possible to decontaminate waste streams containing BTs before disposal. For details, see B12 above. No waste streams containing BTs shall be disposed of in sinks or general refuse. Surplus BTs or chemical waste streams contaminated with BTs 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 BTs 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 BTs 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 BTs 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 BTs shall be handled in accordance with the NIH radioactive waste disposal system.
4. Storage: Store solid BTs and their solutions in dark-colored, tightly closed containers under refrigeration. Do not freeze unstabilized solutions. Avoid exposure to light and moisture.

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

Reviews of analytical methods have been published by Hatheway and McCroskey (1981), Notermans et al. (1982), Das Gupta (1983), and Sakaguchi (1983).

1. Sampling: No data.
2. Analysis: The most common method is bioassay by intraperitoneal or intravenous injection in mice. The classic method is that of Boroff and Fleck (1966) developed for crystalline type A who, using intravenous injection, found a linear relationship over a wide range in a log-log plot of concentration vs. survival time. Three mice per assay point were found sufficient if an accuracy of $\pm 14\%$ is acceptable. Sakaguchi et al. (1968) have applied this method to type E toxin with detectable limits of 5,000 mouse LD50s per ml of test fluid. More recent methods include electroimmunodiffusion which is fast and sensitive (140 mouse LD50s per ml) (Miller and Anderson, 1971) and enzyme-linked immunoabsorbent assay (ELISA), using either "single sandwich" or (preferably) "double sandwich" techniques. This method is equally applicable to cell cultures and pure proteins, and far more sensitive than previously mentioned methods (see Notermans et al., 1982 for applications to types A, B, and E).

Biological Effects (Animal and Human)

Introductory note: This document is not considered to be the place for a discussion of the etiology of human and animal botulism for which standard medical texts should be consulted; it is also reviewed by Lewis (1981), Simpson (1981), and Sakaguchi (1983).

1. Absorption: BTs produce toxic effects by oral and parenteral administration, the former being the usual mode of infection from tainted foodstuffs, or by wound infection by spores of the organism, this probably being the most prevalent form of infection in infant botulism. Absorption after oral administration appears to be from all portions of the intestinal tract. While there is no evidence for transportation across the blood-brain barrier, there may be transplacental absorption since (for type A) feeding of fetuses from infected pregnant rats to mice produced a high incidence of deaths (Hart et al., 1965).
2. Distribution and pharmacokinetics: There is little or no information on distribution; very few experiments have been carried out with radiolabeled BTs, and those few have been criticized (Simpson, 1981) as not distinguishing between active and inactivated toxin molecules.
3. Metabolism and excretion: No information; in particular, the place of dissociation of the large molecules into the active neurotoxin fragments have not been defined. Presumably the pathway is the usual one of proteolysis.

Toxic effects: Toxicity of BTs is usually expressed in terms of mouse LD50s per kg, and it is difficult to translate such data into the more commonly used toxicity figures of mg of protein per kg of body weight. For type A it has been estimated (Bonventre, 1979) that there are 4×10^7 mouse LD50s per mg protein which translates to an LD50 value of 0.025 ng of protein, or less than 10^8 molecules of toxin per 20 g mouse. There are considerable differences in toxicity according to route of administration and to animal species: for instance, for crystalline type A toxin 5 mouse LD50s will kill a 500 g guinea pig via the intraperitoneal route, while 700 mouse LD50s are required for the oral route. The oral toxicity in monkeys is 650 mouse LD50s per kg. Swine and dogs appear quite resistant to BTs (Schantz and Scott, 1981).

The neurotoxins produce their toxic effects through paralysis of skeletal muscle, particularly those of the respiratory system. This paralysis is produced by interference with transmission at the peripheral cholinergic motor nerve terminals of the parasympathetic nervous system. The mechanism of inactivation consists of at least three consecutive steps:

- a. binding of the m.w. 150,000 neurotoxin to cell surface receptors, a rapid, essentially irreversible step which leaves the toxin partially accessible to inactivation by antitoxin,
- b. translocation of the m.w. 50,000 moiety probably by an active process such as creation of protein channels, and
- c. intracellular reaction to inhibit excitation-secretion coupling (Simpson and Das Gupta, 1983; Sathyamoorthy and Das Gupta, 1985).

Paralysis does not set in until the third step is completed.

The above description applies to all neurotoxins with the exception of the type C₂ toxin which is also chemically different from the other neurotoxins (see B3). This toxin does not produce paralysis but exaggerated respiratory movements, tracheal secretions, and pulmonary hemorrhage (Simpson, 1982, 1984). When injected intradermally it produces vascular permeability in guinea pigs (Ohishi et al., 1980b). As mentioned before, both components of the neurotoxin are required for toxic action but these may be administered separately within two hours of each other. It is assumed that the heavier component binds to tissues and makes them vulnerable to the action of the lighter one (Simpson, 1982).

Carcinogenic effects: None have been reported.

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. 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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