

Biotoxins: Part 4

On June 12, 2002, President George W. Bush signed into law the Public Health and Safety Act of 2002 (PL 107-188), which requires that the Department of Health and Human Services maintain a list of biological agents, and toxins which pose a severe threat to public safety. The list of biotoxins, as it appears in the August 23, 2002 Federal Register, (see also 42 CFR Part 72, Appendix A) is as follows:

- Abrin
- Botulinum neurotoxins
- Clostridium perfringens epsilon toxin
- Conotoxins
- Diacetoxyscirpenol
- Ricin
- Saxitoxin
- Shigatoxin and Shiga-like toxins
- Staphylococcal enterotoxins
- Tetrodotoxin
- T-2 toxin

All of these biotoxins with the exception of shiga toxin, shiga-like toxins, and staphylococcal enterotoxins have been topics of earlier PEAC Newsletter Articles. Shiga toxin, shiga-like toxins, and staphylococcal enterotoxins are fairly high molecular weight proteins that are produced by microorganisms and are responsible for some kinds of food poisoning. These proteins can also be aerosolized, or potentially be aerosolized and used as a weapon.

Shiga toxin and Shiga-like Toxins

Description:

As the name implies, we are not dealing with a unique toxic molecule but a class of toxins produced by several kinds of microorganisms. Shiga toxin is produced by the bacterial species *Shigella dysenteriae*. Shiga-like toxins are produced by other bacterial species, mostly by some *Escherichia coli* ["E. coli"] strains. The different toxic protein molecules called "Shiga toxin" or "Shiga-like toxins" differ slightly in their amino acid sequence, but are all characterized in that they inhibit cell protein synthesis. The toxin requires specific receptor sites on the host cell surface in order to enter the cell membrane. Many animal species including pigs and cattle lack these receptor sites and therefore are unaffected by the toxin, but still pass the toxin and the bacteria in their feces. Unfortunately, human cells have these receptor sites and can get very sick if they ingest food products contaminated with these bacteria or toxins. Examples of food products have included undercooked beef, hamburger, pork, unpasteurized fruit juice, contaminated water, spinach, lettuce, and alfalfa sprouts. Person-to-person spread via

fecal or oral transmission may occur in high-risk settings such as day care centers and nursing homes.

Symptoms of Shiga-type food poisoning include diarrhea, which is often bloody, and abdominal cramps. There may be abdominal pain, vomiting, and a low-grade fever. Complications can occur, including damage to kidneys, lungs, and the nervous system. Blood in the urine can occur (hemolytic uremic syndrome, or HUS). In the absence of complications, recovery takes place after several days. Depending upon the circumstances, the incubation period from the time of ingestion to onset of symptoms may vary from hours to about eight days, with 3 to 5 days being typical.

Additional information on HUS including treatment is in a peer-reviewed article available from the Internet, <http://www.emedicine.com/EMERG/topic238.htm>, written by William Shapiro, MD, Department of Urgent Care and Emergency Medicine, Scripps Clinic and Research Foundation. Most cases of HUS in the United States occur with young children (5 years or younger), and about 75% of these cases are associated with Shiga-type toxin food poisoning produced by *E. coli* type O157:H7 infections. About 85% of children recover if given supportive care. The mortality rate is 5 to 15%. The prognosis for recovery for adults is poor, but the incidence of HUS among adults due to Shiga-type toxins is less common. With adults, HUS is more likely to be associated with other causes (other infectious agents, AIDS, administration of chemotherapeutic agents such as mitomycin C, malignancies, etc.)

Thrombocytopenic purpura (TTP) is a complication of exposure to Shiga-type toxin. Cell injury occurs throughout the body including the brain, skin, intestines, skeletal muscle, pancreas, spleen, adrenals, and heart. HUS is the result of cell injury to the kidneys, and is a manifestation of the same overall disease condition. Hemorrhagic colitis (HC) refers to cell damage to the intestinal track.

About 73,000 laboratory-confirmed *E. coli* type O157:H7 infections occur annually in the United States (1999 data, from <http://www.ncbi.nlm.nih.gov>). About 10% of these result in TTP and/or HUS. The O157:H7 strain of *E. coli* is the most common cause of Shiga-type toxin poisoning, but about 100 other strains of *E. coli* can produce toxins. There may be many more cases of food poisoning that are undiagnosed.

At this time, there is no vaccine or antitoxin available for human use. Treatment is basically supportive.

Diagnosis:

The finding of grossly bloody stools in children often with the absence of fever is a strong indicator of *E. coli* infection. The major risk factors are eating insufficiently cooked hamburgers or contact with cattle or other animals that harbor the *E. coli*. With adults, other conditions may result in bloody stools. A postdiarrheal HUS or TTP condition is also a strong indicator of Shiga-type toxin poisoning.

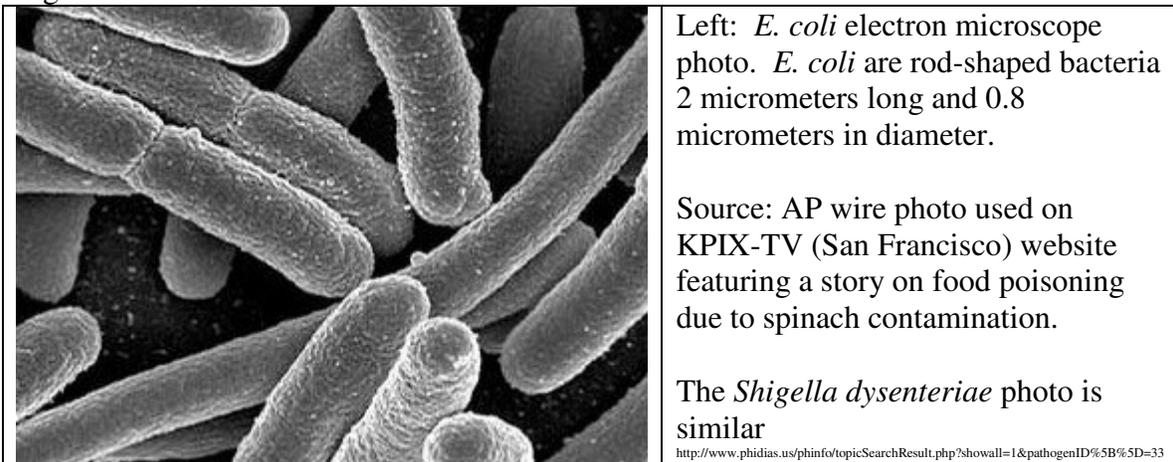
The U.S. Center for Disease Control (CDC) [see http://www.epi.state.nc.us/epi/gcdc/manual/casedefs/E.COLI%20INFECTION-SHIGA%20TOXIN%20PRODUCING_CD.pdf] has published procedures for diagnosis:

- Isolation of *E. coli* strain O157 from a clinical specimen (probable case)
- Identification of an elevated antibody titer to a known Shiga toxin (confirmed case)
- A clinically compatible case that is epidemiologically linked to a confirmed or probable case.

The stx EIA Shiga toxin enzyme immunoassay is the most common procedure used to confirm cases.

Public health authorities in all states recommend that clinical laboratories forward Stx EIA positive specimens of stool samples to the state laboratory for isolation and identification of the Shiga toxin-producing *E. coli* and report to the CDC. A South Dakota laboratory reported (see <http://www.usd.edu/med/path/research/shigatoxin.cfm>) that a toxin-producing strain of *E. coli* was isolated in 89% of the Stx EIA positive specimens, and of these *E. coli*, 54% were strain O157 and 46% were other strains.

Figure 1: *Escherichia coli* bacteria



The Shiga Toxin Molecule:

The total molecular weight of this protein molecule is about 68,000 Daltons. The toxic subpart (A subunit) is about 32,000 molecular weight. The rest consists of five sections (B subunit) each of about 7700 molecular weight, and is responsible for binding to a particular cell type. Once the toxin enters the host cell, the A subunit splits into two parts; the A1 component binds to the ribosome of the host cell disrupting protein synthesis. This kills the cells causing breakdown of the vascular endothelium lining and hemorrhage. The B subunit binds itself to specific glycolipids on the host cell, in particular globotriaosylceramide (Gb3), which is present in greater amounts in renal epithelia tissues (kidneys).

Verotoxin is an alternative name for Shiga toxin and Shiga-like toxin.

The LD₅₀ (mouse) value for Shiga toxin and Shiga-like toxin is less than 20 micrograms per kilogram, by either intravenous or intraperitoneal injection. The LD₅₀ for aerosol exposure is 3 mcg/kg. [mcg = millionth of a gram; per kilogram of body weight].

Shiga toxin produced by *Shigella dysenteriae* differs by only one amino acid from Shiga-like toxin 1 (Stx-1) produced by some *E. coli* strains. Shiga-like toxin (Stx-2), produced by some strains of *E. coli*, differs from Stx (from *Shigella dysenteriae*) by a different amino acid sequence, and is 400 times as toxic (mouse injection) than Stx-1.

Potential for Terrorist Use:

We have not found any confirmed terrorist use, [see <http://www.cbwinfo.com/Biological/Toxins/Verotox.html>], but the toxic protein is robust and easy to manufacture. Both *Shigella dysenteriae* and *E. coli* (strain O157:H7 and other strains) can be easily cultured in the laboratory. The bacterial genes responsible for producing the toxin have been cloned, and the toxin can be manufactured using research strains of *E. coli*.

The toxin is readily soluble in water and can be aerosolized. The toxin does not act through the skin, but is toxic by inhalation. Presumably a full face mask fitted with a charcoal filter should provide adequate protection against aerosolized toxin.

In 1999, a scientist in Germany received a request from North Korea asking that his laboratory furnish them with plasmids used to produce non-toxic fragments of shiga toxin. The scientist was a group leader at the European Molecular Biology Laboratory, and had just published a paper with his colleagues on protein export in cells using the shiga toxin. The scientist did not honor the North Korean request, and a warning was subsequently posted in EMBO Reports on the dangers of possible transfer of the means for development of toxic weapons, <http://www.nature.com/embor/journal/v1/n4/full/embor555.html>. In particular, scientists must adhere to article III of the United Nations Convention on the prohibition of the development, production, and stockpiling of bacteriological and toxic weapons [see <http://www.state.gov/www/global/arms/treaties/bwcl.html>], which obligates the countries “not to transfer to any recipient whatsoever, directly or indirectly, and not in any way to assist, encourage, or induce any State, group of States or international organizations to manufacture or otherwise acquire any agents, toxins, weapons, or means of delivery...”.

Staphylococcal enterotoxins

Description:

Staphylococcus is a name for a microorganism. Enterotoxin means that the toxin produced by the microorganism exerts its effects on the intestines. If allowed to grow in foods, enough toxin may be produced to result in food poisoning after the foods are ingested. The most common symptoms of staphylococcal food poisoning, which usually begin 2 to 6 hours after the food is ingested, include nausea, vomiting, acute prostration, diarrhea, and abdominal cramps. Unlike Shiga-like toxin, the diarrhea is not bloody (unless there is some other pathological condition present), and the onset of symptoms is sooner.

Patients may be dehydrated depending upon the severity of nausea, vomiting, and diarrhea. There may be non-localized abdominal pain.

Most enterotoxins causing human food poisoning are produced by *Staphylococcus aureus*, but several other *Staphylococcus* species have been demonstrated to produce enterotoxins, including *S. intermedius*, *S. hyicus*, and *S. epidermidius*. About 14 different enterotoxins have been identified to date as distinct entities (types A, B, C1, C2, C3, D, E, G, H, I, J, K, L, M, N, and O and the list is growing), of which at least seven are produced by *S. aureus*. The toxin TSST-1, or “toxic shock syndrome toxin-1”, also appears in the literature, and may have originally been called type F toxin. The different toxins are usually called “Staphylococcal Enterotoxin A”, “Staphylococcal Enterotoxin B”, or SEA, SEB, SEC1, ..., SEJ.

Staphylococcus aureus is present almost everywhere. At least 50% of healthy people harbor these microorganisms naturally in their nasal passages and on their skin. Food handlers can easily infect food products. *Staphylococcus* readily grow in unrefrigerated meats, dairy, bakery products, fish, chicken, canned mushrooms, potato, and foods containing eggs. It is one of the most common causes of food poisoning. The actual incidence Staph-caused food poisoning is unknown because many cases are mild and people do not seek treatment, or if the patient visits an emergency room, diagnoses are usually presumptive, and many other conditions mimic the symptoms of Staph-caused food poisoning.

The threshold amount of enterotoxin for causing food poisoning symptoms in humans is not known. There have been some food outbreak studies and human challenge tests indicating that at least 100 nanograms of Enterotoxin A (the most common type of Enterotoxin involved in food poisoning) is required to produce illness [from citations in <http://www.cfsan.fda.gov/~ebam/bam-13a.html>, Bacteriological Analytical Manual Online, U.S. Food and Drug Administration]. The amount and type of enterotoxin is determined by analysis of the toxin in the food (ELISA Assay, Micro-slide double diffusion methods, see citations for procedures). Oral doses of 20 to 500 micrograms of Enterotoxin B produce emesis (vomiting) in nonhuman primates.

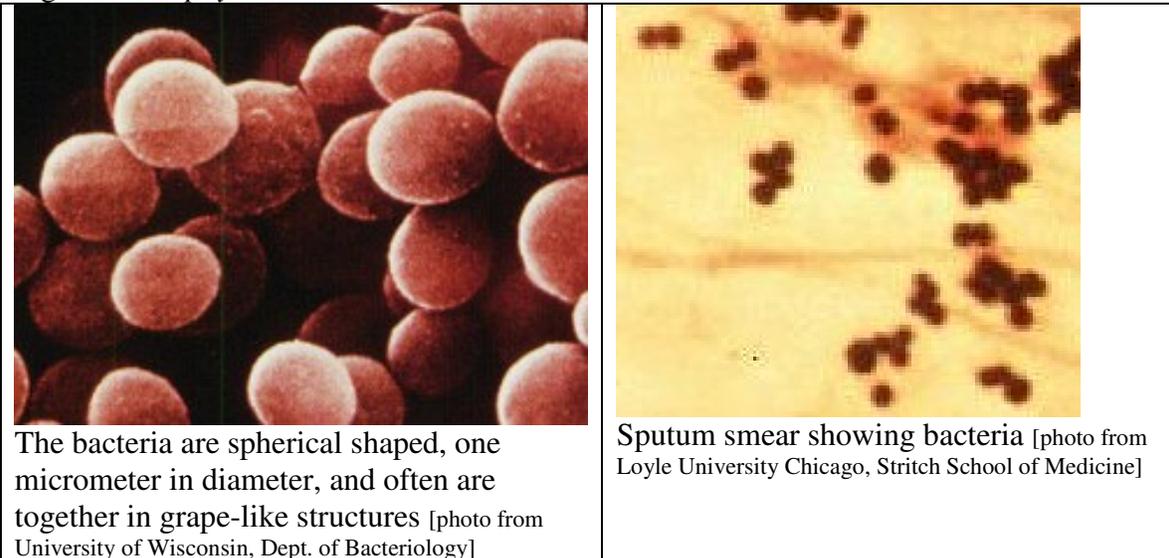
The biological mechanism by which the Staphylococcal enterotoxins act on the body is complex and not fully understood. The enterotoxins are very compact and stable molecules that are not cleaved by enzymes in the intestinal tract. The enterotoxin molecules bind to and crosslink certain cell receptors (HLA-DR or DQ and T-cell receptors) resulting in the production of inflammatory cytokines; therefore the observed

symptoms of nausea, vomiting, and diarrhea. The condition is rarely fatal, and patients almost always recover. Death from this type of food poisoning has occurred with infants, the elderly, and severely debilitated persons.

Treatment is supportive. Persons already in poor health or persons unable to maintain their own hydration require hospitalization.

The enterotoxin does not penetrate human skin and can be removed using soap and water (good hand washing).

Figure 2: *Staphylococcus aureus* bacteria



Additional reading:

- Growth and survival of *Staphylococcus* and toxins in cooking and storage of chicken, in Appl Environ Microbiol, 2006 November; 72(11): 7057–7062, available at <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1636142>
- Safety of Pasteurized Chilled Food. About 90% of *Staphylococcus aureus* is destroyed in food at temperatures of 140°F (60°C) for 5 to 8 minutes, or essentially 100% at temperatures above 180°F, but the toxin itself is heat resistant under normal cooking methods. This article also gives safe holding times and temperatures for previously pasteurized foods, and temperatures required to kill different kinds of bacteria and to deactivate toxins. See International Journal of Food Microbiology, 22 January 2001, pages 99-107, available at <http://www.hi-tm.com/Documents/Chillfd.html>.

If bacteria are allowed to grow and produce toxin in previously prepared food, cooking or re-cooking may kill the bacteria but not deactivate the toxin. The enterotoxin is heat resistant under ordinary cooking methods, and the enterotoxin can remain intact in food even after boiling for 30 minutes at 100°C. Cooking at temperatures of 100°C (212°F) for more than 2 hours is required to destroy the toxin. A University of Wisconsin test [see

Journal of Food Science 57(3) 1992 available at <http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-2621.1992.tb08076.x>], where mushrooms were inoculated with 100 to 1000 nanograms of SEA per gram of mushrooms and then heated to 121.1°C for 10 minutes still showed detectable amounts of SEA after the heating.

Human production of antibodies in response to *Staphylococcus aureus* enterotoxins (SEA, SEB, SEC, SED, TSST-1) has been implemented as a cause of persistent allergic upper airway/nasal/sinus rhinitis and/or asthma conditions [see <http://content.karger.com/ProdukteDB/produkte.asp?Aktion=ShowPDF&ProduktNr=224161&Ausgabe=229964&ArtikelNr=76833>]. The bacteria are present in the airway or nasal passages and produce toxins.

Diagnosis:

Staphylococcal food poisoning is usually diagnosed by clinical symptoms. The toxin can be detected by enzyme-linked immunosorbent assays (ELISA Assays) and chemiluminescence (ECL). A reverse passive latex agglutination test may identify the toxin rapidly in food. Polymerase chain reaction assays can sometimes find *Staphylococcus aureus* genes in environmental samples. If the toxin is inhaled, the toxin can be found in respiratory secretions or nasal swabs for a short period of time, as well as in blood and urine samples.

The enterotoxin molecule:

The molecular weight of the staphylococcal enterotoxins vary from about 23000 to 29600 Daltons. Enterotoxins A, D, and E form one group of closely related toxins (SEA, SED, SEE) and Enterotoxins B, C1, C2, and C3 (SEB, SEC1, SEC2, and SEC3) form a second group. The different toxins vary in molecular weight and amino acid sequencing. SEB, for example, has a molecular weight of 28000 Daltons (one reference says 28,336) and consists of 239 amino acid residues. SEA has a molecular weight of 27,000 and has 233 amino acid residues. Toxic shock syndrome toxin (TSST-1) form a third group, although analysis indicates that the three dimensional structure is similar to SEB. All are compact molecules that are not cleaved by intestinal enzymes. The differences between these types depend how they bind to host cell receptors, with additional binding mechanisms unique to each toxin, and resulting different degrees of toxicity.

The U.S. CDC estimates that SEA accounts for 53% of the Staphylococcal enterotoxin food poisoning. SEB seems to be the second most common type. SED is another common type, typically associated with *S. aureus* growing in dairy products, and probably accounts for 5% of food poisoning. Also, more than one enterotoxin type can be produced by a given bacterial culture.

The lethal dose (LD₅₀) for humans for inhalation of Staphylococcal Enterotoxin B (SEB) is 0.0004 mcg/kg.

The effective dose (ED₅₀) for incapacitating 50% of exposed human populations for SEB inhalation is 0.02 mcg/kg.

A “mcg” is one millionth of a gram, and 0.02 mcg/kg means 0.02 mcg per kilogram of body weight.

[information on dose from <http://www.emedicine.com/EMERG/topic888.htm>]

Potential for Terrorist Use:

Staphylococcal Enterotoxin B (SEB) was weaponized as an incapacitating agent by the United States during the 1960's. President Nixon in 1969 ordered the CIA to destroy the entire stock of biotoxins and biological warfare agents collected over the years and not engage in additional covert research. (see http://www.aarclibrary.org/publib/church/reports/vol1/pdf/ChurchV1_8_Exhibits.pdf for details).

Other enterotoxins can be weaponized, but SEB is the enterotoxin which has been studied.

When inhaled as a respirable aerosol, SEB causes severe respiratory distress, fever, headache, and sometimes nausea and vomiting. The mechanism of incapacitation is due to a massive release of irritating cytokines, caused by binding of the toxin to specific cell receptors. The onset of symptoms may occur in about 2 to 12 hours after exposure and last up to two weeks. At low inhalation doses, symptoms include sudden onset of headache, fever (103° to 106°F, which lasts up to 5 days, if significant pulmonary involvement is involved), myalgia (muscle pain), shortness of breath, nonproductive cough (which may last up to 4 weeks), and retrosternal pain. At high inhalation doses, the illness may progress with increasing respiratory distress, hypoxia (inability to supply oxygen to body tissues), and ultimately respiratory failure due to inflammation and pulmonary edema (fluid buildup in the lungs). Higher levels may lead to septic shock, multi-organ failure, and death.

Treatment (from SEB exposure due to inhalation) is supportive. The basic treatment includes use of humidified oxygen. If exposure is significant, intubation and assisted ventilation with high oxygen concentration may be dictated. Attention should be made to the elimination of hypotension and hypoxia, and pain control as needed. Cough suppressants and acetaminophen for fever will make the patient more comfortable [from emedicine.com, in article by Danielle M. Pesce, DO, at the Carl R. Darnell Army Medical Center]. The administration of antibiotics could make the patient's condition worse.

More detailed studies on inhalation of SEB including a case history investigation of nine laboratory workers who accidentally inhaled SEB is in a paper by Robert Ulrich, et. al, “Staphylococcal Enterotoxin B and Related Pyrogenic Toxins”, and is available from the Internet at <http://www.au.af.mil/au/awc/awcgate/medaspec/Ch-31electrv699.pdf>.

Additional information is available at <http://www.cdc.gov/ncidod/eid/vol10no9/04-0250.htm>.

The bacteria can be readily grown. SEB is stable and is water soluble. Freeze-dried toxin can be stored for over one year. The toxin is moderately resistant to temperature fluctuations, and can withstand boiling at 100°C for several minutes [reference emedicine.com, cited earlier].

There is potential for a terrorist release of the toxin as an aerosol or a mist or a dust.

A military chemical protective mask or a full piece face mask fitted with carbon cartridges is effective against inhalation of the toxin.