Biotoxins: Part 2

The December 2007 AristaTek Newsletter examined the biotoxins, Saxitoxin and Ricin. These were two biotoxins which appeared in a list of toxins which the U.S. Department of Health considered as a severe threat to public safety. This list, as it appeared in the August 23, 2002 Federal Resister, (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

Conceivably, a terrorist could potentially harvest and purify these biotoxins and use them against an enemy or the public. Congress passed the Public Health and Safety Act of 2002 (PL 107-188), and the President signed it into law on June 12, 2002, which required the U.S. Department of Health to maintain a list of biotoxins and biological agents that a terrorist might use.

This Newsletter looks at Abrin, Botulinum neurotoxins, and Clostridium perfringens epsilon toxin.

Abrin

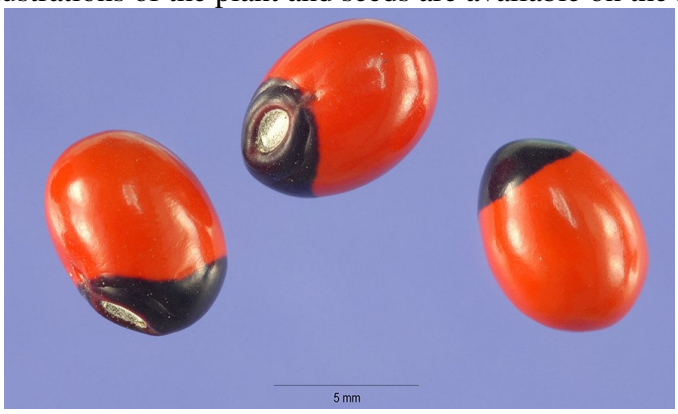
Abrin is a potent toxin extracted from the seeds of the Jequirity Pea (*Abrus precatorius)* plant. The adult fatal dose for abrin is about 0.0026 mg if ingested, or about 0.04 micrograms per kilogram (μg/kg) of body weight. The fatal dose can vary between individuals. Its toxicity is roughly 75 times that of ricin. It is also fatal by inhalation or by absorption through sensitive skin areas as in the eyes.

Like ricin, the toxin acts by inhabitation of body protein synthesis. Initial symptoms by ingestion include watery diarrhea at first, later nausea, vomiting, abdominal cramps, and chills. The vomiting and diarrhea becomes bloody. Severe dehydration may result followed by low blood pressure. Other symptoms may include hallucinations, seizures, and blood in the urine. Within days, the person's liver, spleen, and kidneys may stop working. Death could take place within 36 to 72 hours of exposure. If death has not occurred within 5 days, the person usually recovers but may suffer long-term organ damage. There is no specific antidote. If abrin powder is inhaled, pulmonary edema and hemorrhaging can result. Abrin dust in air can result in blindness or at minimum severe eye irritation. There have also been several reports of people who have ingested the

seeds and slipped into a coma, a condition called “acute demyelinating encephalitis” [see http://www.ncbi.nlm.nih.gov/pubmed/17357388 and Clin. Toxicol (Phil, PA) 2007, 45 (1) 77-79 for a case study, the patient died]

The plant itself goes by many names, including: Rosary Pea, Jequirity Pea, Jequirity Bean, Crab’s Eye, Deadly Crab’s Eye, Precatory pea, Precatory bean, Roseary Pea, Abrus seed, Jumble beans, Ratti seeds, Prayer beads, Tentos de America, Tentos dos mundos, Jequirite, Aivoeiro, Buddist rosary bead, Ruti, Indian bead, and Wild liquorice. The plant itself is a vine which grows up to 10 feet tall and has clusters of rose pink or purplish flowers about 0.5 to 0.8 inches long. The flowers produce brown seedpods with shiny red and black seeds about 5 to 8 mm long. The plant itself is grown in tropical and subtropical areas and has been planted in warmer parts of the United States. The red and black seeds are sometimes used in necklaces and other decorative items.

Illustrations of the plant and seeds are available on the Internet.



Seeds of jequirity pea, about 0.7 cm long
photo by Steve Hurst @ NRCS PLANTS Database

jequirity plant vines

Seeds and seed pod with leaves
Photo by Mark Skinner, USDA NRCS NPDC

Leaf detail, each leaflet 1.2 to 1.8 cm long

One seed if chewed can kill, especially a child. All parts of the plant are toxic to some degree, but the seed, which contains abrin, is especially toxic. If the dried seed is swallowed whole, it is less toxic but some of the toxic elements can still leach out by the

digestive enzymes. Sucking on the seeds can release some of the toxic contents. Immature seeds are poisonous if ingested even whole. If holes are drilled in the seed as in a necklace and the seed is ingested, the toxic contents will leach out by intestinal secretions.

Five glycoproteins have been purified from the seeds, four of which (abrin a, b, c, and d) are extremely toxic. The other glycoprotein is "abrus agglutinin" which is a powerful hemagglutinator but is relatively non-toxic to animal cells; its molecular weight is approximately 134,900.

Arbin (a, b, c, and d) are the toxic parts. The molecular weight is between 63,000 and 67,000. Abrin has been given CAS# 1393-62-0. Purified arbin is a yellowish-white powder. Most of its toxicity is destroyed if heated to 80°C (176°F) for 30 minutes.

The U.S. Department of Health and Human Services – Centers for Disease Control (CDC) Website http://www.bt.cdc.gov/agent/abrin/erc1393-62-0.asp provides the following information about first responder response to an Abrin incident:

Table 1 CDC Emergency Preparedness and Response for Abrin

Exposure	Symptoms	Personal Protective Equipment	First Responder Response
Inhalation	Irritation (see ingestion for other symptoms)	Pressure demand, self-contained breathing apparatus (SCBA) (SCBA CBRN, if available) is recommended in response to non-routine emergency situations. Breath Response (pressure demand) HEPA PAPR. See CDC/NIOSH Interim Recommendations.*	Fresh air, rest, half-upright position. Perform CPR if necessary. Seek medical attention immediately.
Skin	Potential for allergic skin reaction; redness, blisters, pain. May be absorbed	Tychem® BR or Responder® CSM protective clothing. Eyes should be protected when possible.	Remove contaminated clothes. Rinse skin with plenty of water or shower (and soap if available). Seek medical attention immediately.
Eyes	Tearing, swelling of the eyelids, pain, redness, corneal injury.	Goggles with respiratory protection or full face-piece respirator.	Immediately flush with large amounts of tepid water for at least 15 minutes. Seek medical attention immediately.

*http://www.cdc.gov/niosh/unp-intrecppe.htm

Table 1 continued

Exposure	Symptoms	Personal Protective Equipment	First Responder Response
Ingestion	• Cardiovascular (heart and blood circulation) shock from severe dehydration, life-threatening low blood pressure, fast heart rate (tachycardia) and irregular heart rhythms (arrhythmias). • Central Nervous System (brain) - drowsiness, disorientation, hallucinations, seizures, coma. • Gastrointestinal (stomach and intestines) - burning pain in the mouth, abdominal pain, nausea, vomiting, diarrhea, bleeding and swelling of the lining of the GI tract, liver cell damage and death. • Genitourinary (kidneys and urine) - blood in urine, low or no urinary output, kidney cell damage and death. • Musculoskeletal - muscle weakness, tremors, and muscle spasm (tetany) • Eyes - dilated pupils and bleeding in the back of the eye (retinal hemorrhage) • Skin - blue skin (cyanosis) and redness (flushing) • Symptoms delayed 1-3 days. • May be fatal.	Do not eat, drink, or smoke during work. Wash hands before eating.	Rinse mouth. Do not induce vomiting. Use slurry of activated charcoal. In the event of vomiting, lean patient forward or place on left side (head-down position, if possible) to maintain open airway and prevent aspiration. Early and aggressive IV fluid and electrolyte replacement. Seek medical attention immediately.

As far as we have been able to determine from an Internet search, Abrin is not known (to date) to have been used in any wars or terrorist attacks. Abrin also has potential medical uses such as in treatment to kill cancer cells.

Additional reading:

1. Summary, to Congress, bioterrorism report, 2003

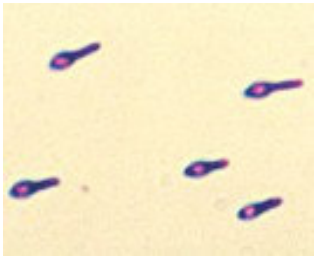
http://www.fda.gov/oc/bioterrorism/report_congress.html

2. EY Laboratories Inc test kit for Abrin and Ricin

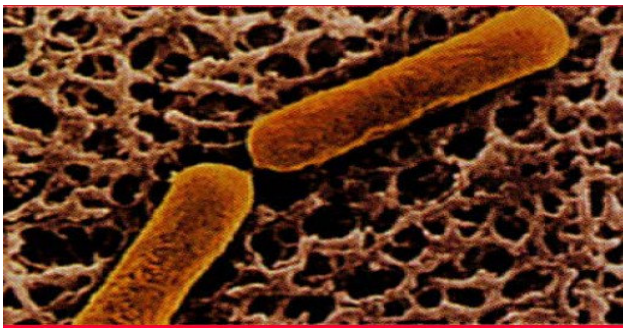
http://www.eylabs.com/index.php?page=shop.product_details&category_id=140&flypage=shop.flypage_new&product_id=2210&option=com_virtuemart&Itemid=79&vmcchk=1

Botulinum neurotoxins

One of the most toxic biotoxins known is botulinum neurotoxin, with an estimated human lethal dose of 0.001 micrograms per kilogram of body weight (µg/kg) by inhalation, or 1 µg/kg if ingested. The poisoning is called botulism. There are actually seven types of botulinum neurotoxins recognized, labeled serotypes A through G, with type A being the most toxic and types B and E causing less severe toxicity to humans. The botulinum neurotoxins are produced primarily by a spore-forming bacterium known as *Clostridium botulinum* and to a lesser extent by spore-forming bacteria *Clostridium baratii* and *Clostridium butyricum*. *Clostridium sporogenes* does not produce the neurotoxin. These bacteria and its spores are found worldwide in soil, ponds, costal waters, sediments, the intestinal tracks of fish and mammals, and in shellfish.



CDC photo of *Clostridium botulinum* stained with a dye (Gram Stain), spore pink and rest of bacterium stained purple



Detail of unstained *Clostridium botulinum* bacterium, length 3 to 8 µm, spore diameter (not visable in photo) about 0.5 µm., photo from http://www.maphtc.iupui.edu/html/CD_Training/Bioterrorism/video/BTDentalTeam/Agents.pdf

Human botulism is primarily caused by types A, B, E, and F neurotoxin. Types C and D most commonly affect fowl, birds, cattle, and horses and do not affect humans. Type E typically occurs from eating contaminated fish. Type G, isolated from a soil in Argentina, has not yet been linked to any outbreak. All of them are single polypeptide chains (molecular weight about 150,000) that work by blocking the release of acetylcholine from peripheral cholinergic nerve endings. What this means is that the nerve terminals are blocked, and the muscles do not work, usually starting at the eyes and face, then the throat, chest, and extremities. When the diaphragm and chest muscles become fully involved, respiration is inhibited and death occurs. There are differences on how the different types interact with nerve endings and block the release of acetylcholine, which is the reason why some types are more toxic than others or are more toxic to bird or animal species or to humans.

The neurotoxin itself is destroyed if heated to 80^{o}C (176^{o}F) for 10 minutes or longer. If heated to 100^{o}C (212^{o}F), destruction occurs quicker. However, cooking food containing spores does not destroy the spores at 100^{o}C (212^{o}F). When canning food, the spores must be destroyed by moist heat at temperatures of at least 121^{o}C (250^{o}F). The spores if ingested normally do not prorogate and produce neurotoxin in adult humans because gut

conditions are too acidic, but the spores can colonize and produce neurotoxin in the intestinal tracks of infants under 12 months of age.

Four different kinds of botulism poisoning are recognized:

1. Inhalation botulism. This kind does not occur naturally and could mean that a terrorist has released the toxin as an aerosol.
2. Food borne botulism. This is usually the result of ingestion of food that has been inadequately processed or inadequately cooked before being eaten. If food is contaminated with *Clostridium botulinum* spores, and the bacteria allowed to grow under anaerobic conditions, the neurotoxin will be produced. If not cooked properly to destroy the toxin, botulism food poisoning will occur. The most common source is home-canned foods. There have been incidences of poisoning in commercial products. Neurological symptoms usually appear within 6 hours to 8 days of ingestion of the food, the shorter time associated with more severe poisoning.
3. Infant (intestinal) botulism. This results from ingestion of bacteria spores which germinate in the intestinal gut and produce the toxin. This can happen as the result of food, soil, or dust contaminated with the spores. The infant gut is most susceptible to spore germination, but adult infections can occur if acidic conditions are not maintained in the gut. This is the most common incidence of botulism poisoning reported to the CDC in the United States. Pediatricians do not recommend feeding honey to infants as the honey could contain the spores.
4. Wound botulism. This happens when wounds become contaminated with dirt containing botulism spores. This is especially a problem with chronic injection drug users. The incubation period is between 4 and 14 days since contamination, the shorter time corresponding to the more severe infection. Neither the spores nor the neurotoxins are able to penetrate intact skin.

About 110 cases of U.S. botulism poisoning are reported to the CDC annually.

Conformation of botulism poisoning is done by either detection of botulinum toxin or by isolation of *Clostridium botulinum* in a clinical specimen and the patient also displays clinical symptoms of botulism poisoning. The specimen could be a person's stool sample in the case of infant or food borne botulism or a serum sample, or a food sample. The standard test for botulinum toxin testing is the mouse neutralization test.

A sensitive method for detection of botulinum neurotoxin in foods has been published recently (see **http://aem.org/cgi/content/abstract/72/2/1231** for abstract). The citation is S.K. Sherma, J. L Ferreira, B.S. Eblen, and R.C. Whiting, "Detection of Type A, B, E, and F *Clostridium botulism* Neurotoxins in Foods by Using an Amplified Enzyme-linked Immunosorbent Assay with Digoxigenin-Labeled Antibodies", Applied and Environmental Microbiology, 72(2), Feb. 2006, p. 1231-1238. The enzyme-linked immunosorbent assay (ELISA) test is an alternative test to the mouse neutralization test.

Antitoxin therapy is available and is administrated to adult patients with food borne or wound or inhalation botulism. The antitoxin must be administrated early. It is not

administrated to infants. The CDC must release and approve its use. The antitoxin is a horse serum product and may cause serum sickness in approximately 20% of treated persons. A human-derived antitoxin product is under evaluation in California (see World Health Organization Poisons Information Monograph 858) for infants. Also, the decision to administer the antitoxin often can't wait for conformation by testing for the toxin or bacteria, and must be made on the basis of display of symptoms especially if a suspect food is identified or there is evidence of a wound which may have been contaminated. The patient's serum should be collected before administration of the antitoxin.

The specific antitoxins available from the CDC are

- Licensed Bivalent Anti-AB equine antitoxin, Aventis-Pasteur
- IND Univalent Anti-E equine antitoxin, Aventis-Pasteur
- IND Heptavalent despeciated (Fab'2) equine Anti-ABCDEFG antitoxin, U.S. Army

California Dept. of Health Services, Infant Botulism Treatment and Prevention Program

- Licensed Anti-AB human antitoxin for infant botulism "Baby-BIG"

(reference: University of Alabama School of Medicine website at **http://www.bioterrorism.uab.edu/CategoryA/Botulinum/summary.asp**)

The classic symptoms of botulism poisoning include double vision, blurred vision, drooping eyelids, slurred speech, difficulty swallowing, dry mouth, and muscle weakness. Infants appear lethargic, feed poorly, are constipated, have a weak cry, and have a poor muscle tone. If untreated, these symptoms may progress to cause paralysis of the arms, legs, trunk, and respiratory muscles. (from CDC website, at **http://www.cdc.gov/ncidod/dbmd/diseaseinfo/botulism_g.htm**.)

Because of respiratory failure, the patient may need to be on a breathing machine (ventilator) for weeks. If severe, the person may require intensive medical and nursing care for months.

The person affected by botulism poisoning is not infectious.

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Several countries are reported to have produced botulinum neurotoxins as weapons. The United States once produced botulinum neurotoxin under military code name X. The entire stockpile of botulinum neurotoxin was ordered destroyed by President Nixon (1969) along with other biological agents. The former Soviet Union continued their biological weapons research program. In April 1992, President Boris Yeltsin declared that his country had continued a massive offensive biological warfare buildup which included botulinum warfare buildup. In 1995, Iraq admitted to the United Nations Special Commission inspection team that it had produced 19,000 liters of botulinum neurotoxin concentrate for use in specially designed missiles and sprayers, including approximately 10,000 liters loaded in military weapons. In 1990, Iraq deployed 13 missiles with a 600 km range containing botulinum toxin. (source: "The Comprensive

Sourcebook of Bacterial Protein Toxins" (book), edited by J.E. Alouf and M.R. Popoff, Academic Press, 2006).

Fortunately, botulinum neurotoxins are unstable in the environment. Therefore the range of an aerosol attack out in the open is limited. The neurotoxin would be expected to degrade in the environment within one or two days. Contaminated surfaces can be cleaned with a 0.1% chlorine bleach solution.

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Additional reading: (from Journal of American Medical Association, ***JAMA*. 2001;285:1059-1070**):

http://jama.ama-assn.org/cgi/content/full/285/8/1059?maxtoshow=&HITS=10&hits=10&RESULTFORMAT=&fulltext=botulism&searchid=1049721556467_1604&stored_search=&FIRSTINDEX=0&journalcode=jama

The University of Florida Environmental Health and Safety Manual lists LD_{50} values for each of the Botulinin toxins, which can be obtained at **http://www.ehs.ufl.edu/Bio/toxin.htm**.

Table 2 LD_{50} values for Botulinin Toxins, [Univ. of Florida, citing D.M. Gill, Microbiological Reviews 46: 86-94. (1982) and other sources]

Toxin	**LD50 (µg/kg)**
Botulinin toxin A	0.0012
Botulinin toxin B	0.0012
Botulinin toxin C1	0.0011
Botulinin toxin C2	0.0012
Botulinin toxin D	0.0004
Botulinin toxin E	0.0011
Botulinin toxin F	0.0025

Clostridium perfrigens epsilon toxin

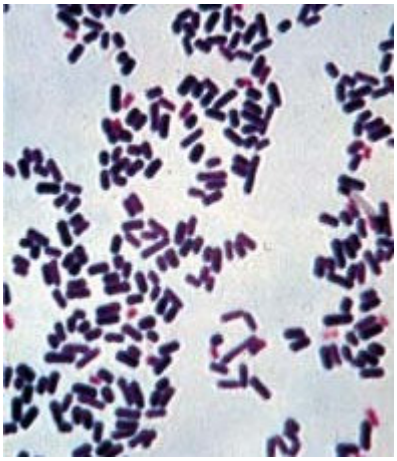
Clostridium perfrigens is the name of the microorganism and epsilon toxin is one of several biotoxins that it produces. Gas gangrene results from wound contamination by this microorganism, which grows in the wound and produces biotoxins. In addition, *Clostridium perfringens* can cause food poisoning. Clinical symptoms resulting from food poisoning include intense abdominal cramps and diarrhea which begins between 8 to 24 hours after eating food containing these bacteria. The toxins are a particular problem with farm animals, in particular, goats, sheep, young caves, and pigs. Theoretically, a person could also become poisoned by inhalation of dust containing the microorganism or its toxin. The toxins can be transmitted in contaminated food, water,

or as an aerosol. Specific information on human poisoning by the epsilon toxin is minimal, but farm animals are affected.

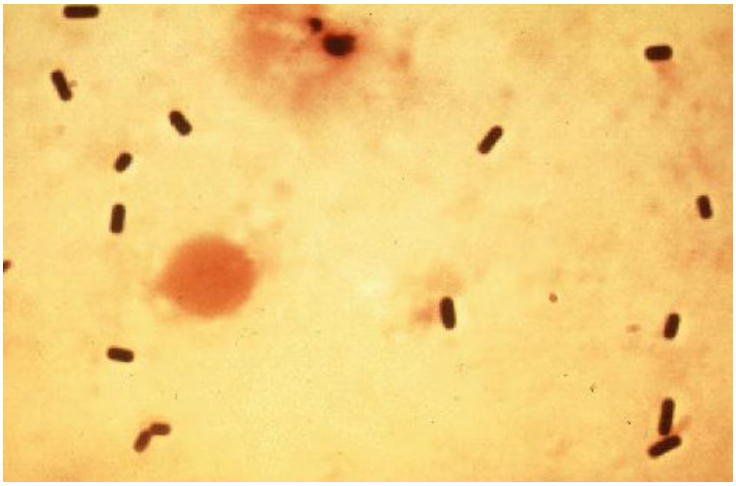
There are 5 different strains of *Clostridium perfringens* (labeled Types A, B, C, D, and E) and perhaps at least 20 toxins (labeled alpha, beta, beta2, etc, epsilon, iota, etc.; some sources cite 12 toxins). The toxins vary in toxicity and in their effect on animals and people. The epsilon toxin (which is produced by strains B and D) is singled out as being the most toxic, based primarily on intervenous injection of the toxin to test animals. The LD_{50} value (lethal dose resulting in death of 50% of the test animals) for the epsilon toxin using a mouse as the test animal is 0.78 nanograms (0.1 µg/kg of body weight), (ref: Iowa State University, Center for Food Security and Public Health website). Goats and lambs injected with the epsilon toxin develop severe pulmonary edema (fluid in their lungs) and show neurological symptoms. Rats injected (intraperitionally) develop cerebral edema. Onset of neurological symptoms by intervenous injection may occur anywhere from 2 minutes to one hour in test calves to 0.5 hours to 3 hours for goats and lambs. The epsilon toxin works by causing potassium and fluid leakage from body cells. Neurological symptoms include stupor (mild poisoning) to coma and death (more severe poisoning).

Table 3 LD_{50} values for ***Clostridium perfringens*** Toxins, [Univ. of Florida, citing D.M. Gill, Microbiological Reviews 46: 86-94. (1982) and other sources]

Toxin	**LD50 (µg/kg)**
Clostridium perfringens epsilon toxin	0.1
Clostridium perfringens beta toxin	0.4
Clostridium perfringens lechithinase toxin	3
Clostridium perfringens delta toxin	5
Clostridium perfringens perfringolysis O	13 to 16
Clostridium perfringens enterotoxin	81
Clostridium perfringens kappa toxin	1500



Clostridium perfringens **gram positive bacterial stain, anaerobic, rod-shaped**



Tissue smear showing *Clostridium perfringens*

Both photos from Kirksville College of Osteopathic Medicine website, at http://www.geocities.com/capecanaveral/3504/gallery.htm

About one-third of the incidences of food poisoning in the United States is caused by *Clostridium perfringens,* mostly from the type A strain producing the alpha toxin, or enterotoxin. A more serious form is the type C strain producing the beta2 toxin. Other strains besides type A produce the alpha toxin. Symptoms (from the alpha toxin) of food poisoning include diarrhea, nausea, severe abdominal cramps, and bloating but rarely vomiting or fever. The symptoms appear 8 to 24 hours after ingestion of contaminated food. Patients generally recover within a day or two, although the elderly can take longer. Deaths are rare. However, the type C strain producing the beta2 toxin can cause sloughing of the mucosa off the intestines and intestinal perforation, and severe infection, often resulting in death. Symptoms include a bloody stool and probably vomiting and fever in addition to type A symptoms. Intervention including removal of part of the intestine with prolonged antibiotic treatment and intravenous fluid replacement may be necessary. This type of food poisoning may be called "necrotizing enteritis" or "pig-bel disease". The beta2 toxin is usually associated with enteritis in pigs, therefore the name "pig-bel disease".

Information on human food poisoning from the epsilon toxin is sketchy and is complicated by proper identification of the strain and maybe more than one toxin can be produced. Fluid replacement including electrolyte monitoring is part of treatment as potassium loss is a feature of epsilon toxin poisoning. Limited information available on the Internet seems to suggest that the symptoms are similar to that produced by the alpha toxin.

The purified alpha toxin can be lethal by inhalation as an aerosol, causing acute pulmonary disease, vascular leak, hemolysis, thrombocytopenia, and liver damage.

The toxins including the epsilon toxin can be destroyed by heat. Thorough cooking of food is required. The vegetative form of the bacteria can be destroyed at 70^{o}C (158^{o}F), but much higher temperatures and prolonged heating is required to kill the spores. The high temperatures required to kill all of the spores also compromise the nutritional value of the product, and sometimes food processors use a combination of gamma irradiation treatment and heat, or add salt or other preservatives. When cooking meat, it is also important to cool the meat product down quickly and refrigerate if consumed at a later time. More details are at a University of Wisconsin, Food Research Institute website, **http://www.wisc.edu/fri/briefs/cperfsurvivgrow.pdf**, in a paper published in 2002, © Food Research Institute, "Survival and Growth of *Clostridium perfringens* during the Cooling Step of Thermal Processing of Meat Products".

Toxin identification in humans and animals can be done using the mouse neutralization test (MNT) or enzyme-linked immunosorbent assay test (ESILA) on clinical samples (e.g. feces, intestinal fluids). Another test is the polymerase chain reaction assay test (PCR).

An Internet search failed to turn up evidence that Chostridium perfringens epsilon toxin has been weaponized. The South African biological weapons program between 1981 and

1984 developed the bacteria as a potentially lethal agent to resemble food poisoning. In 1991, Iraq representatives informed the United Nations Special Commission inspection team that Iraq had researched the offensive use of *Chostridium perfringens* as a potentially lethal agent, and had produced 90 gallons of the bacteria.

Presumably the toxin can be manufactured by fermentation of *Clostridium perfringens* or more likely by another microorganism expressing the cloned gene for the toxin. The microorganism. *E. coli* has already been cloned to produce the epsilon toxin for the production of a vaccine for inoculation of farm animals. The purified toxin or even the crude material could be released as an aerosol. A person inhaling the material would be expected to suffer severe pulmonary edema (fluid buildup in the lungs) in addition to vascular leak, organ damage, and neurological problems including cerebral edema, as the result of potassium leakage from body cells. The alpha toxin is also a potentially lethal agent when dispersed as an aerosol.

Additional information on biotoxins may be found at the website, http://www.globalsecurity.org under Weapons of Mass Destruction.

There is a good overview article on toxins formed by ***Clostridium*** bacteria published by the University of Wisconsin (2008), Madison Department of Bacteriology, at http://www.textbookofbacteriology.net/clostridia.html.

[Author's disclaimer: In searching the Internet, I found some discrepancies between LD_{50} values cited in different sources, and also which ***Clostridium sp.*** toxins are more toxic. Also there are discrepancies as to the number of toxins produced by ***Clostridium perfringens.*** I have not resolved these differences by searching out the original publications where the LD_{50} work was done]