

Biotoxins

Biotoxins are toxic chemicals produced in nature by living things. The living things may be bacteria in the animal gut, certain plants which produce potent toxins such as ricin, spore-forming bacteria producing anthrax toxin, red algae producing saxitoxin, certain animals and insects producing toxic venom, etc.; the list is long. A terrorist or a nation state can potentially harvest and purify a toxin to be used against an enemy, perhaps by poisoning a water supply or by releasing a dust/aerosol in an airplane passenger compartment or inserting a fine powder in a letter. However, most poisonings occur accidentally when people eat contaminated food or from contact with venomous creatures.

Two of the natural biotoxins are classified as “Schedule 1 Chemical Warfare Agents” under the United Nations agreement on biological weapons, e.g. the Chemical Weapons Convention in 1993 and earlier agreements. These are saxitoxin and ricin. These are not the only potent biotoxins. We will look at saxitoxin and ricin in this newsletter.

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

Saxitoxin

Saxitoxin is a potent toxin which if ingested (or injected) works as a selective sodium channel blocker in the nervous system resulting in rapid, incurable paralysis and death. A dose of 0.2 milligram is fatal to an average-weight human being. Saxitoxin is produced by “red-tide” algae, one of the algae species “*Alexandrium tamarense*.” This algae species is found in marine waters throughout the world. The usual mode in which humans ingest this toxin is from eating contaminated shellfish (clams, mussels, oysters), which in turn have ingested (“filtered”) the algae at some previous time in their life, even though the “red tide” may have past and the waters look clean. Cooking the shellfish does not destroy the toxin.



Red tide off California coast, *Noctiluca sp.*, photo by PJS Franks, from Woods Hole Oceanographic Institution photo gallery

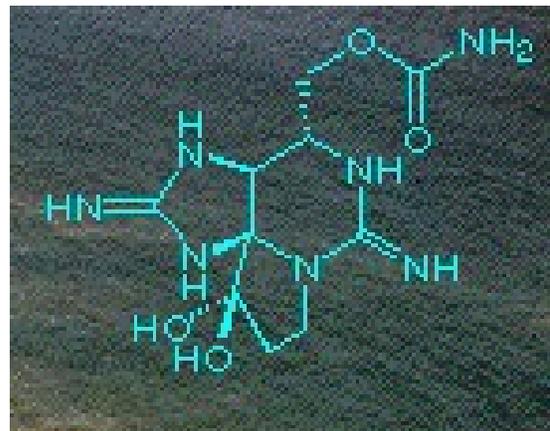


Red tide off New Zealand coast, photo by M. Godfrey, from NIWA Science website

Another photo of “red tide” is in the May 2008 month of “Toxic by Nature” calendar issued jointly by Drug Discovery & Development and Gilson Inc. More photos are at <http://www.whoi.edu/redtide/rtphotos/rtphotos.html>



Alexandrium sp. cyst, from Woods Hole Oceanographic Institution



Chemist's representation of Saxitoxin., photo from University of Sussex at Brighton, UK.

In the 1950's the CIA began experimenting with the biotoxin. Saxitoxin was produced and stockpiled in the United States as part of a chemical weapons program at Fort Detrick, MD using the code name Agent TZ. It is not clear what the CIA involvement was, but Internet sources suggest development of dart guns and manufacture of suicide tables for their agents if captured. Agent TZ is soluble in water and can be ingested or inhaled. If an intended victim is jabbed with a dart tip, death occurs quickly.

President Nixon in 1969 ordered the CIA to destroy its entire stock collected over the years and not engage in additional covert research with saxitoxin (see http://www.aarclibrary.org/publib/church/reports/vol1/pdf/ChurchV1_8_Exhibits.pdf for details). In 1975, the CIA director revealed to Congress that they still possessed 10

grams of saxitoxin, in violation of the 1969 presidential order, which was then evenly distributed to scientists and medical researchers under the auspices of the National Institute of Health. Saxitoxin is useful in the study of nerve disorders because it selectively blocks only the sodium channels but does not affect potassium or calcium channels or the chloride ion count or acetylcholine response.

In 1977, a paper was published in the open literature (Kishi et al, Journal of the American Chemical Society, 1977, vol 99, page 2818) on the chemical synthesis of saxitoxin. The synthesis was modified in another paper published in 1984 (Jacobi et al., Journal of the American Chemical Society, 1984, vol 106, page 5594) to yield an optically pure product mimicking what is produced in nature. The Jacobi synthesis was carried at Wesleyan University in Connecticut.

Saxitoxin is said to be 1000 times more toxic than the nerve gas Sarin. The 0.2 milligram fatal dose for an average weight human is based on LD50 mice studies (the lethal dose resulting in the death of 50% of the test animals in 24 hours). The LD50 for mice is 8 microgram/kg, but humans are 4 times more sensitive to than mice to oral doses of saxitoxin because the human digestive track is longer and more saxitoxin is absorbed. The lethal oral dose for humans is 1 to 4 mg depending upon age and physical condition. Children are apparently more sensitive than adults.

Alexandrium tamarense is not the only algae species that produce biotoxins. The causative algal species mostly belong in the general classification of dinoflagellates, which include *Alexandrium tamarense*, *Alexandrium circinalis* (a fresh water species), *A. minutum*, *A. ostenfeldi*, *A. catenella* (Pacific coast), *A. fundyense* (northeastern U.S. and Canadian coast), *Gymnodinium catenatum*, *Karenia brevis* (eastern Gulf of Mexico), various *Dinophysis sp.* (Europe, Asia, Japan), and *Pyrodinium bahamense* (Philippines and elsewhere). The affected waters may be red, grey, brown or other colors, e.g. “brown tide”, or the waters may not be noticeably colored. At least 12 different biotoxins are produced from various dinoflagellate species and have also been studied. The biotoxins fall under the general chemical classification of tetrahydropurines, of which saxitoxin is the first studied and best known. There are also other biotoxins besides tetrahydropurines produced from algae blooms.

No human deaths have been directly attributed to direct contact to “red tide” algae, but people may experience respiratory irritation when winds blow aerosol onshore from waters containing “red tide” algae. Skin irritation and burning is possible when swimming in areas affected by “red tide” algae. Deaths of people and animals (including birds, fish, turtles, etc.) occur because of consumption of mollusks and other creatures, which feed on the algae; the mollusks concentrate the biotoxin in their flesh as the result of their filter feeding. The biotoxins have resulted in deaths of fish, marine animals, sea turtles, and birds. Generally, fish and shrimp caught in “red tide” waters are safe to eat at least in smaller quantities (check with the local health department/authorities to be sure). However, the 1987 deaths of 14 humpback whales off Massachusetts in Cape Cod Bay were traced to the whales eating mackerel, which in turn had eaten smaller fish and zooplankton, which had consumed large amounts of *Alexandrium tamarense*. The

saxitoxin was concentrated in the food chain. The 1987 death of 700 bluenose dolphins was also similarly traced to bioaccumulation of neurotoxins in their fish diet.

The method used by the CIA at the chemical weapons program at Fort Detrick, MD involved harvesting a certain species of clam grown in “red tide” waters, and extracting the saxitoxin from the clams.

Four types of human shellfish poisoning have been identified:

- Paralytic shellfish poisoning (PSP)
- Amnesic shellfish poisoning (ASP)
- Neurotoxic shellfish poisoning (NSP)
- Diarrhetic shellfish poisoning (DSP)

Table 1. Example Biotoxins from Eating Shellfish

Biotoxin	Algae species	Poisoning Type	Fatal dose
Saxitoxin (CAS 35554-08-6)	<i>Alexandrium sp.</i> and certain other dinoflagellates	PSP	0.2 mg for average weight human. 100% recovery from non-fatal doses without brain damage
Brevetoxin (There are several toxins)	<i>Gymnodinium breve</i> (Gulf of Mexico and Caribbean), <i>Karenia sp.</i> and other dinoflagellates	NSP	Deaths rare, but patients may suffer dementia. Rat and human studies show brain damage.
Domoic acid (CAS 14277-97-5)	<i>Nitzchi pungens</i> (a diatom) implicated in 1987 Canadian outbreak. Also <i>Pseudonitzschia sp.</i> (east and west coast of U.S. and Gulf of Mexico)	ASP	Rat LD50 (injected subcutaneously) 0.33 mg/kg. In a 1987 Canadian outbreak, of 145 patients studied, the three patients who died were elderly and showed severe damage to the hippocampus and other parts of the brain. For 10 cases, from mussel analysis, a 4.2 mg/kg oral dose of toxin resulted in severe neurological effects.
Okadaic acid (CAS 78111-18-8)	<i>Dinophysis sp.</i> ; also <i>Prorocentrum lima</i>	DSP	Deaths rare, but elderly patients experience memory loss. Large dose of okadaic acid from contaminated mussels results in permanent neurological sequelae. LC50 (mice, injected subcutaneously) 0.192 mg/kg

PSP description: The onset of symptoms occurs within 5 to 30 minutes after ingestion of contaminated shellfish. Initially there is a slight tingling progressing to numbness around the mouth, neck, and face. In severe cases, these symptoms spread to the extremities in coordination and respiratory difficulty. There may be difficulty swallowing, sense of throat constriction, speech incoherence, headache, dizziness, nausea, possible vomiting, and reduced eye pupil size. In severe cases, within 2 to 12 hours, there is complete paralysis and death from respiratory failure in the absence of ventilatory support. Without artificial respiration, up to 75% of severely affected patients, die within 12 hours. Gastric lavage and administration of activated charcoal or dilute bicarbonate solution is also recommended. Benzedrine is effective in aiding artificial respiration. The PSP symptoms mimic acute organophosphorous pesticide or nerve gas Sarin poisoning, but the use of anticholinesterase agents is not recommended for PSP patients and may do more harm than good. After about 12 hours, if death has not occurred, patients start to recover gradually and are without residual symptoms after a few days.

Cooking the shellfish does not destroy the biotoxin.

It is imperative to obtain samples of the shellfish tissue and their source so that diagnosis can be made. The mouse bioassay of the food extract is the usual diagnostic method. Radioimmunoassay and indirect enzyme-linked immunosorbent assay have been developed for saxitoxin, but not all of the biotoxins, which cause PSP. HPLC analysis methods have been developed for all of the PSP toxins.

NSP description: There are several different kinds of brevetoxin (given names such as brevetoxin A, brevetoxin B, and other names). Brevetoxins act by disrupting the flow of sodium ions in nerve cells. They bind to the sites near the nerve cells allowing an unchecked flow of sodium ions in and out of the cells (in contrast to saxitoxin which binds different sites and blocks sodium ions from passing through the sodium channel). Brevetoxin poisoning rarely results in death, and patients recover within a few days, but permanent nerve damage and dementia can occur. Symptoms include false temperature sensations, muscular aches, dizziness, and anxiety. These are usually accompanied by vomiting, diarrhea, and abdominal pain. Cooking the shellfish does not destroy the biotoxin.

ASP description: Much of what is known is the result of investigation of 153 cases of acute intoxication, which were reported in 1987 as the result of individuals eating mussels harvested from Prince Edward Island, Canada. The onset of symptoms varied between 15 minutes to 38 hours after eating. Many of the patients were elderly and suffered gastrointestinal distress and also neurological effects that included memory loss and dementia. Younger patients seemed to have more digestive problems. Twenty-three (23) patients required intensive care because of seizures, coma, profuse respiratory secretions, or unstable blood pressure. Three patients died. The cause of death was coma, encephalopathy, convulsions, and cardiovascular collapse. In the mouse bioassay, mice were given intraperitoneal injections of extracts from the mussels. The mice soon exhibited an uncontrolled scratching of both shoulders with their hind legs, and most of the mice died within 3.5 hours after injection. Further research demonstrated that the toxin was domoic acid, an amino acid with a molecular weight of 311. The involved mussels contained between 31 and 128 mg of domoic acid per 100 grams of mussel tissue. The toxin was produced by an algae (diatom) called *Nitzschia pungens*, which in turn was ingested by the mussels during their normal filter feeding. In ten patients studied, there was a clear dose response between amount of domoic acid in mussels consumed and neurological effects. Further tests using monkeys using mussel extracts gave similar dose response to the ten patients studied. Later research demonstrated the presence of domoic acid in anchovies in California, and razor clams and crabs off British Columbia. Domoic acid caused the death of large numbers of Brown pelicans and cormorants in 1991 and over 400 sea lions in 1998, both incidents off the California coast. The anchovies in California had been eating the diatom, *Pseudonitzschia australis* that produced the domoic acid; the seabirds that died in 1991 in turn ate the anchovies.

In the brain, domoic acid damages the hippocampus and amygdaloid nucleus. It damages neurons by activating AMPAS and kainatic receptor causing an influx of calcium. The uncontrolled increase of calcium causes the nerve cells to degenerate. When the

hippocampus is damaged, long-term memory loss occurs. There is no antidote for domoic acid.

Cooking the mussels does not destroy domoic acid.

DSP description: Acute high-dose exposure to okadaic acid, which accumulates in certain clams and some crabs, is the underlying cause of human DSP. Symptoms occur between 30 minutes to 12 hours after eating contaminated shellfish. Symptoms include diarrhea, nausea, vomiting, abdominal cramps, and chills. Gastrointestinal bleeding and hiccups have occurred. Full recovery is usually experienced within a few days, but death can occur especially in elderly patients due to coma, seizures, and/or pulmonary edema. DSP mostly occurs in Europe, Japan, and South America. Over 5000 people experienced DSP in Spain in 1981. Okadaic acid (and/or its esters) primarily affects the cells lining the intestinal gut; the exact mechanism is not clear, but the chemical is thought to stimulate phosphorylation that controls sodium secretion by intestinal cells and also affects calcium ion transport in general across cell membranes in the body. Patients over 50 years old may also experience neurological effects including memory loss, severe anterograde amnesia, and motor or sensorimotor neuropathy.

Cooking the shellfish does not destroy the biotoxin.

References: Most of the information presented here on shellfish poisoning came from (1) a paper prepared by Charles Baier, University of Idaho, December 2000, "Red Tide and Shellfish Poisoning: Toxic Products of Marine Algae" which is available at http://www.agls.uidaho.edu/etox/resources/case_studies/REDTIDE2.PDF and (2) from the United States National Library of Medicine TOXNET at <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB> .

Ricin

Ricin is a toxin extracted from the castor bean. The (adult) human fatal dose for ricin is 0.2 milligrams if ingested, and about 0.05 milligrams if injected. It is also fatal by inhalation. Children are more sensitive than adults. Ricin acts by inhibition of protein synthesis. Symptoms appear within a few hours after ingestion. The initial symptoms are abdominal pain, vomiting, and diarrhea (sometimes bloody). Within several days there is severe dehydration, urine decrease, and drop in blood pressure. If death has not occurred within five days, the victim usually recovers but suffers long-term organ damage. A castor bean might pass through the gut unscratched if its surface is not broken (as in chewing), but eight chewed castor beans contain enough ricin to kill an adult and one chewed castor bean can kill a child. There is no specific antidote.

The castor bean plant (*Ricinus communis*) originally is a native of tropical Africa. It is now grown worldwide, sometimes as an ornamental in gardens or as a houseplant, and also grows as a weed in tropical and semitropical areas. The growing of the plant is not illegal, but in the United States (see Title 18, United States Code, part 175) a person

caught manufacturing or possessing ricin may be sentenced up to 10 years, or life for possession with intent to use as a weapon or to provide to a foreign government.

Photographs and descriptions of the castor bean plant and its seed are readily available from the Internet making recognition easy. In tropical areas the plant can grow to almost tree-like size with up to 20-inch leaves. The leaves are usually eight-lobed as shown in the photograph with slightly serrated edges and prominent central veins.



Source: Univ. of Illinois



Source: Cornell

<http://www.ansci.cornell.edu/plants/castorbean.html>



Source: NPR website, photo
Ketzal Levine



Source: Cornell



Source: Wikipedia as used in
USA Today website

Ornamental varieties grown in gardens may be purplish with narrower leaf lobes and smaller like the photo at the left. The flowers may be green or they may be pink or red as shown above (center). The soft flower capsules mature into soft spiked seed capsules each containing several mottled castor beans about 0.4 inch long.

More than one million tons of castor beans are processed each year to make useful products such as castor oil. Besides its use as a laxative, castor oil when dehydrated is used extensively in paints and varnishes, and is said to have qualities superior to linseed oil. Its water resistant qualities make it ideal for coating fabrics and for protective

coverings. Other use is in the production of sebacic acid, which is the basic ingredient in the production of nylon. Castor oil has an ability to cling to very hot moving parts making it an outstanding lubricant for high performance engines.

Ricin does not partition into the castor oil product because the ricin is water-soluble. The ricin stays behind in the seed-pulp left over in oil extraction. The residual seed pulp (also called oil cake, or pomace) may contain up to 5% ricin. The seed cake does make an excellent fertilizer. There are also numerous documented cases of ricin poisoning of horses or other livestock when they accidentally ate castor bean seeds or meal. Castor bean meal mixed in with bait is highly toxic to rodents and insects.

Procedures for extraction of ricin from castor seeds or seed pulp are said to be available on the Internet, and this author has not attempted to research these procedures. The trick is to separate the ricin from the other complex proteins, which form part of the castor bean, and a simple extraction using water (or lye or acetone) plus filtration does not accomplish this. Consequently homegrown recipes are likely to produce a mixture of plant proteins, and the ricin produced might even be partly denatured (inactivated). Another toxin from castor beans called "Ricin communis agglutinin" is a powerful hemagglutinin (clumps red blood cells) but does not penetrate the intestinal wall. U.S. Patent 3060165 (granted in 1962, withdrawn in 2004) may be the basis of some procedures. As mentioned before, manufacture of ricin is illegal.

Mass spectrometry can be used as a method of forensic identification of ricin including ricin in castor bean extracts. The procedure is described by Sten-Åke Fredriksson et al, "Forensic Identification of Neat Ricin and of Ricin from Crude Castor Bean Extracts by Mass Spectrometry", 2005, *Anal. Chem.* (An American Chemical Society publication), 77 (6), pp 1545-1555.

Ricin is toxic by inhalation, injection, and ingestion. It is also absorbed by mucous membranes and the eyes. There is no known antidote.

There have been attempts to weaponize ricin by governments, but because the material is difficult to deploy and is easily deactivated, historically it has not been used as a mass casualty weapon. Historically, specific individuals have been targeted. The potential for use as a mass casualty weapon is there. Al Qaeda has reportedly experimented with ricin (according to an interview with German magazine *Der Spiegel*, cited in wikipedia). There are also a few incidents where powdered ricin has been sent in first class letters in the United States, including one sent to U.S. Senate Majority Leader Bill Frist in November 2003.