Technical Dialogue

Biotoxins Used As Warfare Agents

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A PEAC user called AristaTek, Inc., and asked why the chemical "ricin" was not listed under the PEAC classification of biological warfare agents. Ricin is a very toxic chemical derived from the castor bean and has the potential to be "weaponized", that is, a toxic aerosol of the chemical can be prepared which can be inhaled. The PEAC tool listed "ricin" under the broad classification of chemical warfare agents and not biological warfare agents because it is basically a chemical and not an agent which causes disease. However the PEAC user pointed out that the U.S. Military lists biotoxins under the broad classification of bioterrorist agents along with smallpox, pneumonic plague, and anthrax. Chemicals such as VX, Sarin, and mustard gas are classified under chemical warfare agents. We have a problem with definition.

Definitions:

A "biotoxin" is a poison produced by living organisms. The living organisms might be plants, an animal, fungi, microbes, algae, etc.. Over 400 biotoxins have been identified and many more exist in nature. The list includes ricin produced from the castor bean plant, snake venom, various toxins produced by bacteria, tetrodotoxin isolated from the livers of globe fish, aflatoxin produced by certain molds, cicutoxin from water hemlock, histrionicotoxin from the skin of the poison arrow frog, etc., ; the list is long. However the list is shortened to perhaps 15 or 20 biotoxins if we limit ourselves to those poisons which can be potentially used as a weapon of mass destruction. Only a few biotoxins produced in nature can be mass produced and dispersed in the form of an aerosol which can be inhaled or sprayed onto surfaces and absorbed through the skin. Poisoning of food and water supplies is possible, but the poison would have to be mass produced and added to the food source or water supply. Many biotoxins (but not all) are destroyed by cooking or by normal water chlorination.

Biotoxins which enter the human body do not replicate. Disease-causing microorganisms do replicate themselves. The PEAC tool listings for biological warfare agents are limited to diseases that might be spread by a terrorist or used in warfare. Many disease-causing microorganisms produce biotoxins in the human body. If the disease-causing microorganisms can be grown in the laboratory or mass-produced and a poisonous but non-infectious material isolated, we have listed this material under the classification of a biotoxin. The disease or microorganism causing the disease is classified under biological warfare agents. For example, botulism is listed in the PEAC tool under "biological warfare agents". Botulism is a disease caused by a microorganism (a spore-forming bacterium called *Clostridium botulinum*). The microorganism produces the very toxic botulinum toxin. The bacteria can be grown in fermentors and the toxin harvested. Therefore the PEAC tool also has a listing under chemical warfare agents as "botulinum toxin". The next PEAC tool release will have botulinum toxin listed under the classification of "biotoxin".

How Toxic Are Biotoxins?

Table 1, below, lists LD_{50} values for laboratory mice inhaling selected biotoxins. The LD_{50} value is the dose in units of micrograms per kilogram of body weight that results in 50% kill of the test animal. The information was obtained from David R. Franz, 1997 (revised), "Defense Against Toxin Weapons", U.S. Army Medical Research Institute, Fort Detrick, MD.

Table 1. Comparative Lethality of Selected Toxins in Laboratory Mice

Biotoxin	Source	Molecular Weight	LD_{50} , μg/kg
Botulinum toxin	Bacterium	150000	0.001
Shiga toxin	Bacterium	55000	0.002
Abrin	Rosay Pea Plant	65000	0.04 by inhalation 20 by ingestion
Maitotoxin	Marine dinoflagellate	3400	0.10
Ciguatoxin	Fish/marine dinoflagellate	1000	0.40
Batrachotoxin	Arrow poison frog	539	2.0
Ricin	Castor bean (plant)	64000	3.0
Tetrodotoxin	Puffer fish	319	8.0
Saxitoxin	Marine dinoflagellate	299	2.0 by inhalation 10 by ingestion
Anatoxin-A(s)	Blue-green algae	500	50
T-2 Toxin	Fungal mycotoxin	466	1210

Incapacitation as well as lethality must be considered. Some toxins such as the T-2 toxin cause illness at doses many times less than the concentration required to kill.

By comparison, the LD_{50} value (mice) for the chemical warfare agent Sarin is 100 μg/kg. For the chemical warfare agent VX, LD_{50} is 15 μg/kg. For Soman, LD_{50} is 64 μg/kg. On a per unit weight basis, some biotoxins are more potent than any of the synthetic chemical warfare agents.

How do Biotoxins Act on the Human Body?

Toxins can be classified by the mechanism of toxicity. The two broad classifications are cytotoxins and neurotoxins.

- Cytotoxins cause cellular destruction

- Neurotoxins affect the central nervous system. Neurotoxins may be further classified into (1) presynaptic and postsynaptic neurotoxins, (2) ion-channel and sodium-ion binding toxin, and (3) ionophires.

Neurotoxins block nerve conduction and cause death by paralyzing muscles of respiration.

Ricin is an example of a cytotoxin. T-2 Toxin is a hermorrhagic cytotoxin which causes bleeding. Botulinum toxin and saxitoxin are examples of presynaptic and postsynaptic neurotoxins. Tetrodotoxin is an example of an ion-channel neurotoxin. Ciguatoxin (from fish contaminated with a dinoflagellate) is a sodium-ion binding toxin.

How might a Terrorist Deliver a Biotoxin?

The most likely method an aggressor might target military troops and civilian populations is as a respirable aerosol, which allow the toxin to contact the inner surface of the lung. There are major technological problems that the aggressor must overcome. None of the biotoxins form gases or liquids that vaporize. All of them are solids (or powders) which must be delivered as an aerosol. To be most effective, the aerosol particle should be between 0.5 and 5 microns diameter to be captured by the lung surface. Particles larger than about 20 microns fall harmlessly to the ground. Particles between 5 and 20 microns may lodge in the nasal passages and trachea and therefore are not as effective as when lodged in the lungs. A substantial portion of particles less than 0.5 microns in diameter will be exhaled and not retained in the lungs.

Consider ricin which has a LD_{50} toxicity of 3 μg/kg. Assuming the data obtained from mice also apply to man, a 70 kg man receiving a 3(70)/1000 = 0.21 milligram dose would have a 50% chance of surviving. The amount of ricin required to achieve this dose over a one kilometer square area is estimated to be about 80 kilograms. This assumes that the ricin is emitted as a respirable aerosol near the ground. Even though ricin is very toxic, there are technological problems in covering a large surface area with the toxin.

With botulinum toxin, less quantity is required. Botulinum has a LD_{50} of 0.001 μg/kg. We are now talking of 32 grams of botulinum toxin to cover a one square kilometer area. Again, this is assuming that the right-sized aerosol diameter is produced, the aerosol is delivered uniformly over the area, and meteorological conditions are ideal.

Again, these are idealized, theoretical calculations. Because of inefficiencies in the aerosol delivery system, larger quantities would have to be delivered to achieve an LC_{50} effect.

Some biotoxins result in incapacitating illnesses at levels much below the LC_{50} dose. An example is staphylococcal enterotoxin B which causes illness at very low dosages (vomiting and diarrhea). The LD_{50} by inhalation is 0.027 μg/kg. Staphylococcal enterotoxin B is a cytotoxin that causes the body to release large amounts of its own chemicals and fluids.

Thichothecene mycotoxins in low dosages cause skin lesions and systemic illness without being inhaled and absorbed through the respiratory system. The most likely route of exposure is through the skin. A dose of one-billionth of a gram per square centimeter of skin is sufficient to cause irritation of the skin. A dose of one-millionth of a gram causes destruction of skin cells (necrosis). Microgram dosages can cause irreversible injury to the eye. The LD_{50} dose for one of the mycotoxins (T-2 Toxin) is 1210 μg/kg.

An assassin could target an individual by injecting a biotoxin-contaminated pellet. An example is Bulgarian exile Georgi Markov who was apparently killed by a ricin-containing pellet injected in his thigh in London in 1978.

What about Contamination of Food and Water Supplies?

Fortunately, there are a number of factors that work against massive contamination of public food and water supplies. Normal water chlorination will destroy bacterial toxins, for example, 5 parts per million of chlorine for 30 minutes destroys botulinum toxin. Water chlorination at these concentrations is ineffective against ricin, saxitoxin, T-2 toxin, or microcystin. Cooking food will also inactivate most biotoxins. Coagulation/floculation at a water treatment plant will remove the higher molecular weight compounds/proteins but is ineffective against ricin, saxitoxin, or other lower molecular weight compounds. Carbon adsorption or reverse osmosis water treatment removes biotoxins, even the lower molecular weight compounds. Ingestion of most biotoxins is less toxic than inhalation.

Diagnosis

Responders and health care providers ask whether they may tell the difference between a chemical warfare attack, a biotoxin attack, or a biological warfare attack using infectious agents. Radiation detection equipment is essential for detecting a dirty bomb attack.

The onset of incapacitating symptoms in a chemical warfare attack is almost immediate (within minutes). The onset may be a little longer if the chemical agent is absorbed through the skin. Chemical nerve agent poisoning is a violent illness resulting in respiratory failure, airway constriction, and increased body secretions (saliva and airway secretions), pinpoint pupils, perhaps also convulsions and muscle spasms.

Symptoms are delayed in the case of a biotoxin attack or a biological warfare attack. There may be a delay of several days, even a week, from the time of exposure to an infectious agent to the time symptoms first occur. The delay may be on the order of minutes to two or three days in the case of a biotoxin attack depending upon the toxin and route of exposure. As expected, the delay is less if the toxin is inhaled. It is difficult, in general, to distinguish between a biotoxin and biological warfare agent attack. Diagnosis is based on specific symptoms and conformation by laboratory tests.

Each toxin must be considered individually. Some toxins incapacitate so quickly that there would be little time for therapy after an attack. Fortunately, the potent bacterial protein toxins (e.g. botulinum toxin) act slower, and therapy is usually successful if started within about 12 hours after exposure providing the toxin is identified.

Protection

Biotoxins are solids, usually a powder, which when weaponized is dissolved or slurried in a liquid (usually a water or weak alcohol solution) and then an aerosol created. Tight-fitting face masks can protect emergency response personnel. Eventually the aerosol will settle on the ground or on surfaces. Because toxins are not volatile, they should not pose a further threat (providing they are not ingested). An exception is the situation where the biotoxin also contains an infectious agent such as anthrax spores. Another exception is some mycotoxins (e.g. T-2 toxin) which are absorbed through the skin.

If the toxin contaminates skin and clothing, ordinary soap and water should remove almost all of the toxin.

Ricin

Description: Ricin is a cytotoxin derived from the beans of the castor plant. Although it is roughly a 1000 times less toxic than the most potent of the bacterial toxins on a per unit weight basis, the widespread availability of castor beans and the ease of production make ricin a potential biological weapon. Ricin acts on the human body by preventing protein synthesis.

Symptoms of ricin poisoning: This depends upon whether ricin is ingested or inhaled. Inhalation of an aerosol toxin results in upper airway and pulmonary symptoms which may occur from minutes to about 6 hours after exposure depending upon the dose. Symptoms may include nasal and airway congestion, nausea and vomiting, itching of the eyes, urticaria, and tightness of the chest. After 12 to 24 hours, depending upon the dose, pulmonary manifestations include airway lesions and edema (lungs fill with fluid). Death occurs 36 to 48 hours after exposure. From mice studies, LD_{50} = 3 μg/kg. Ricin is less toxic by ingestion, but ingestion of two castor beans will kill a human. Ingestion results in nausea, vomiting, abdominal pain and cramping, diarrhea, fever and chills, hematochezia, a drop in blood pressure, shock and vascular collapse, but no lung damage. Autopsy findings have found significant hepatic, splenic, and renal necrosis.

Diagnosis of ricin poisoning: Diagnosis is based on clinical and epidemiologic factors. Conformation of ricin exposure by inhalation can be made by ELISA analysis of a swab sample from nasal mucous taken less than 24 hours after exposure.

Treatment: Treatment is supportive. Inhalation injury may require treatment of pulmonary edema with respiratory support. Early following ingestion, patients should undergo GI decontamination, with administration of activated charcoal. Intravenous crystalloid infusion and pressor support may be necessary for patients with hypotension.

Botulinum Toxin

Description: Botulinum toxin is a neurotoxin produced by the microbe *Clostridium botulinum*. This anaerobic, spore-forming bacillus produces on of the most lethal toxins known, the estimated lethal dose to humans LD_{50} = 0.001 micrograms/kg. The toxin can be mass-produced in fermentors and can be aerosolized. Botulism food poisoning can occur by ingestion of food containing the toxin or bacterial spores. The toxin acts on the human body by preventing the presynaptic release of acetylcholine blocking neurotransmission; the result is muscular weakness and paralysis. Several toxin types are known (designated A through G) with type A being the most toxic.

Symptoms: Symptom onset for inhalation typically occurs between 24 to 36 hours after exposure but could be less if the dose is high. Initial symptoms include headache, queasiness, blurred vision, muscle weakness, mydriasis (dilatation of eye pupil), ptosis (drooping of upper eyelid), dysphagia (difficulty in swallowing), and dysphonia (slurred speech). This progresses to increased muscle weakness and eventual respiratory paralysis. Deep tendon reflexes may be depressed or absent on physical examination. Patients may

become cyanotic (skin appears bluish because of carbon dioxide buildup in the blood) secondary to respiratory failure. Ingestion may also result in vomiting and involuntary defecation.

Diagnosis: Diagnosis is based on clinical and epidemiologic factors (see symptoms described above). Confirmation of inhalation intoxication can be made using ELISA analysis of nasal swabs taken less than 24 hours after exposure. Ingestion exposure can be detected by analyzing serum or gastric fluids with a mouse neutralization assay.

Treatment: Treatment is supportive. As most serious complication is respiratory failure, ventilation assistance is necessary. An antitoxin is available for administration in confirmed cases. There are risks associated with administrating antitoxins developed from serum products (e.g. horse serum).

Staphylococcal Enterotoxin B

Description: Staphylococcal enterotoxin B is one of the most common causes of food poisoning. Nausea, vomiting, and diarrhea follow ingestion of contaminated food. It is listed in the PEAC tool as a biotoxin because of the toxin can be mass-produced and is stable as an aerosol which can be inhaled. The inhaled dose necessary to incapacitate individuals is small, about 0.004 micrograms/kg. Individuals usually recover. In severe cases of inhalation exposure, death may occur due to pulmonary edema and toxic shock. The LD_{50} value (by inhalation) is 0.027 micrograms/kg.

Symptoms: Symptoms begin to occur in 2 to 12 hours after exposure. In more severe inhalation cases, symptoms may occur 1 hour after exposure. Mild to moderate inhalation exposure produces nonspecific systemic illness characterized by fever, chills, headache, chest pain, myalgias (muscle pain), and a non-productive cough. Ingestion produces vomiting and diarrhea. More severe exposure results in pulmonary edema and toxic shock. The duration of the illness is 3 to 10 days.

Diagnosis: Diagnosis is based on clinical and epidemiologic factors. Laboratory studies may show a nonspecific neutrophilic leukocytosis and an elevated erthrocyte sedimentation rate. A radiograph may show interstitial edema in patients with significant pulmonary symptoms. The toxin accumulates in the urine and can be detected for several hours after exposure. If the toxin is inhaled, it may be isolated from nasal swabs taken less than 12 hours after exposure.

Treatment: Treatment is supportive, with close attention paid to oxygenation and hydration. Patients with severe toxin exposure may need ventilator support and diuretics.

Collection of Biological Samples (blood, urine, nasal swabs, saliva)

Identifying toxins or their metabolites in biological samples is very difficult because only a very small amount of toxin is required to cause illness. Therefore extremely sensitive assays are required. Furthermore, the biological samples must be collected soon after exposure or the molecules will be lost. The samples must be kept refrigerated until they can be analyzed as the toxin will be destroyed if stored at room temperature. Enzyme-linked immunosorbent assays (ELISA) of nasal swabs are definitive diagnostic tests for inhalation of biotoxins. They are sensitive to about 1 to 10 nanograms per millimeter and require about four hours to complete. The polymerase chain reaction technique provides a very sensitive means of

detecting and identifying the genetic material of any living organism that might remain in the crude, impure toxin collected in the field.