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4. BIOLOGICAL TOXINS

Biological toxins are substances produced by living organisms – bacteria, plants and animals – for defence or predation. One dictionary defines biological toxins as "Chemicals produced by living organisms that have toxic properties for another organism". Biological toxins have been used by humans for a long time. The first information on natural poisons and some guidelines on their preparation can be found in the Ebers Papyrus, which dates back to about 1,500 B.C. This paper looks at many of the plants containing poisonous substances. There are many ways of classifying biological toxins. The first is by their action, into A and B categories. Category A includes neurotoxins that affect the functions of the nervous system with effects that are temporary and theoretically reversible. Category B includes toxins that directly damage cells and whose effect causes functional disorders of tissues and organs. Tissues and organs are damaged indirectly or directly by release of secondary mediators. The effects of such toxins are often irreversible and cause permanent health damage. A biological toxin's lethal potential is measured in terms of the amount of material required to kill 50% of a group of test animals (usually rats or mice). This is written as LD_{50}. LD_{50}<25 mg/kg means that a substance is very toxic; LD_{50}<25 mg/kg to 200 mg/kg is toxic; LD_{50}<200 mg/kg to 2,000 mg/kg <LD_{50} is harmful, while substances of LD_{50}>2,000 mg/kg are not classified as toxic agents. The most potent biological toxins are produce by bacteria, plants and fungi, and some animal toxins also have deadly potential.

Biological toxins are very attractive to terrorists, for use in acts of bioterrorism. The first reason is that many biological toxins can be obtained very easily. Simple bacterial culturing systems and extraction equipment dedicated to plant toxins are cheap and easily available, and can even be constructed at home. Additionally, the actual toxins are easy to obtain and in most cases not restricted by law. Many toxins affect the nervous systems of mammals by interfering with the transmission of nerve impulses, which gives them their high potential in bioterrorist attacks. Others are responsible for blockage of main cellular metabolism, causing cellular death. Moreover, most toxins act very quickly and are lethal in low doses (LD_{50}<25 mg/kg), which are very often lower than chemical warfare agents.

Table 1. Comparison of biological toxins toxicity with chemical warfare agents

Agent	LD_{50} parameter (μg/ml)	Molecular weight
Botulin toxin	0.001–0.002	150,000 (Protein)
Shiga toxin	0.002	55,000 (Protein)
Tetanus toxin	0.002–0.003	150,000 (Protein)
Abrin	0.01–0.04	65,000 (Protein)
Ricin	0.1–1	65,000 (Protein)
Clostridium perfingens toxins	0.1–5	35,000–40,000 (Proteins)
VX	15	267
Staphylococcal enterotoxin B	27	25,000 (Protein)
Soman	64	182
Sarin	100	140
Aconitine	100	647
T-2 mycotoxin	1,210	466

The potential of biological toxins in bio warfare was presented in a report of the Iraq Biological Warfare Program, prepared by the UN Special Commission in 1995. The report concluded that Iraq had 19,000 litres of botulinum toxin and 2,400 litres of aflatoxin at its disposition.

4.1. Bacterial toxins

Bacterial toxins are substances produced and released by bacterial cells to destroy other bacteria and surrounding cells. Most bacterial toxins are proteins, encoded by bacterial chromosomal genes, plasmids and phages. Bacterial toxins can be classified as either exotoxins or endotoxins. Exotoxins are generated and actively secreted; endotoxins remain part of the bacteria. Endotoxins are usually part of the bacterial outer membrane, and are released by lysis of the bacterial cell. The response of an organism to an endotoxin can include severe inflammation. In general, the inflammation process is usually considered beneficial to the infected host, but if the reaction is severe enough it can lead to sepsis. Exotoxins are usually secreted by bacteria and act at a site removed from bacterial growth. Exotoxins are usually proteins, minimally polypeptides, that act enzymatically or through direct action with host cells and stimulate a variety of host responses.

4.1.1. Botulin toxin

Botulin toxin is a neurotoxin produced by the spore-forming, Gram positive, anaerobic bacteria *Clostridium botulinum*. Spores are resistant to heat, chemical substances, radiation and aerobic conditions. One disease caused by the toxin

is botulism. Botulin toxin has a protein construction (with a mass of 150 kDa) and it belongs to a group of metal proteins (zinc-dependent endopeptidases). It is built of two polypeptide chains: a heavy chain with a mass of 100 kDa and a lightweight chain (50 kDa), connected by a disulphide bond. This toxin is not resistant to chemical and physical agents. It is depredated at 85°C in 5 minutes, and is destroyed by sunlight within 1-3 hours. Additionally, it is immediately decontaminated by chloride or H_2O_2.

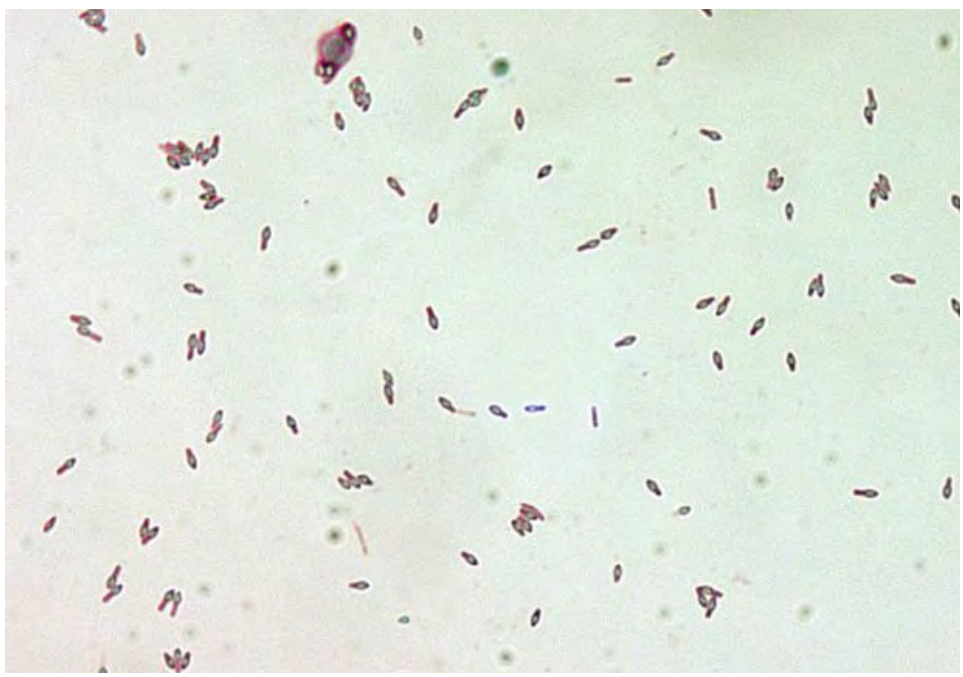

Figure 1. *Clostridium botulinum* spores (*figure used with permission under Creative Commons license*)

The protein has three domains responsible for its biological functions: a binding domain which binds the toxin to the receptor located on the surface of the target cells of the cellular membrane; a translocation domain that transports the toxin through the plasma membrane into the cell, and an enzymatic domain with a proteolytic activity that causes defragmentation of the SNAP-25 protein, which is responsible for merging synaptic bubbles with a cellular membrane and releasing neurotransmitters. The binding and translocation domains are located within the heavy chain, while the domain with enzymatic activity is within the lightweight chain. The mechanism of the toxic action of botulin toxin inhibits the release of neurotransmitters, including acetylcholine, within neuromuscular junctions, resulting in de-contraction and relaxation of skeletal muscles.

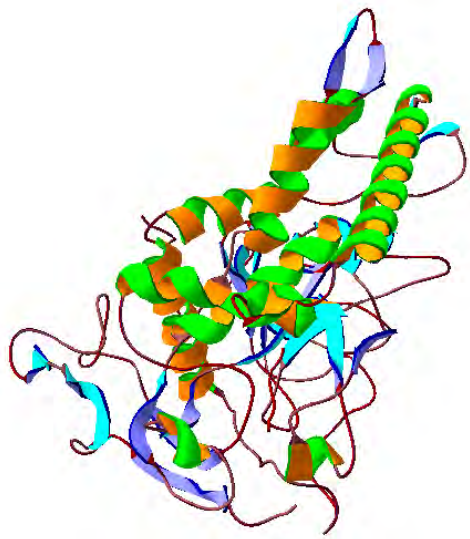

Figure 2. Botulin toxin structure

Botulin toxin is the most toxic substance in the known world, with an LD_{50} of 1-2 ng/kg. As the only biological toxin, it has been classified by the Centre for Disease Control and Prevention (CDC) in Atlanta as a Category A bio agent. The lethal dose for a human weighing about 70 kg is 0.7–0.9 μg of inhaled toxin, or 70 μg of poison ingested with food.

There are 7 kinds of botulin neurotoxin: A, B, C, D, E, F and G. Types A, B, E and F are dangerous to humans, with type A being the most dangerous and most toxic. It can be detected in soil samples from China, South America and the western territories of the United States. Type B toxins can be isolated from the soil of the eastern United States and Europe. Type E can be found in the sea and lake sediments in the northern hemisphere. Types C and D are dangerous to animals, although no cases of disease as a result of poisoning with type G have been reported so far. *Clostridium botulinum* spores are also capable of developing in poorly prepared and stored food. This applies to meat, fish and vegetable preserves.

In nature there are 4 sources of botulism: from food, wounds, animals and in infants. Regardless of where it grows, similar clinical symptoms of botulin poisoning are observed, and all occur within several hours of the toxin's penetration into the body. Initially, the symptoms include difficulty speaking and swallowing, double or unclear vision, anuria, and cessation of saliva and tears. Next, control of one's body is lost, along with the gag reflex. Paralysis of the respiratory muscles causes respiratory insufficiency and this is the main cause of death of infected people. Consciousness and physical sensation are maintained throughout all of the symptoms, which aggravates the suffering. Foodborne botulism occurs after eating poorly preserved

food. Wound botulism is caused by the *Clostridium botulinum* bacteria penetrating the body through broken skin, e.g. wounds suffered in accidents. Most cases of wound botulism are observed in drug addicts taking heroin subcutaneously. Infant botulism occurs through the ingestion of honey or powdered milk contaminated with *Clostridium botulinum s*pores. An analysis of global infant botulism between 1996 and 2008 revealed 524 cases in 26 countries. Botulism in animals is a result of eating feed contaminated with bacteria spores. Horses, cattle, birds and minks are particularly sensitive to botulin toxin.

Treatment of botulism is based on administering botulin antitoxin as quickly as possible. Its role is to neutralise the toxin. Additionally, in most cases mechanically-assisted respiration and supportive treatment (with a return to self-reliance in up to 2–3 months), is also needed.

In a bioterrorist attack, botulin toxin can be used in the form of an aerosol or can be added to food and water sources. The neurotoxin's effectiveness is equally high, regardless of the route of exposure, due to the nearly identical symptoms. Initial symptoms can be mistaken for other symptoms of chemical warfare agents, such as sarin or VX, and this can additionally impede rescue actions. The mortality rate in such attacks is estimated as being nearly 100%. For this reason, the first responders should be prepared to recognize poisoning with this toxin.

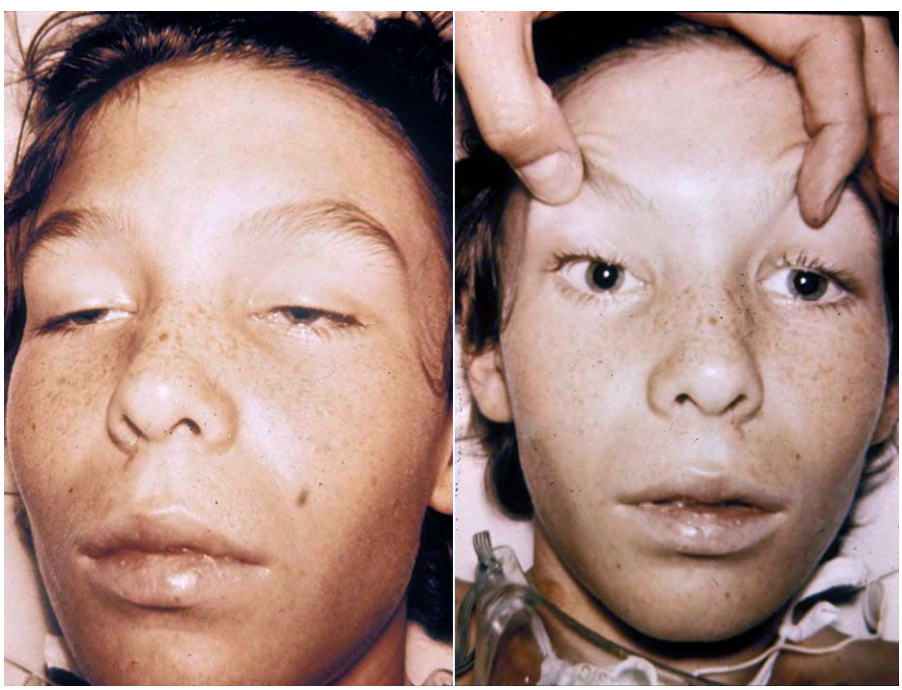

Figure 3. Typical symptom of botulin poisoning – ptosis (*figure used with permission under Creative Commons license*)

Table 2. Comparison of typical symptoms of botulin toxin and nerve gas poisoning

Botulin toxin	Nerve agent
paralysis	convulsions
no change in heart rate	bradycardia
drooping eyelids (ptosis)	constricted pupils (mioz)
increased secretory functions	lack of secretory functions

4.1.2. Tetanus toxin

Tetanus toxin is another potent neurotoxin produced by the vegetative cells of *Clostridium tetani* in anaerobic conditions, which causes tetanus. Similarly to *Clostridium botulinum*, this bacteria also forms very resistant spores. In nature, *Clostridium tetani* is found in soil, especially heavily-manured soils, and in the intestinal tracts and faeces of various animals. The toxin is produced during cell growth, sporulation and lysis. The LD_{50} of this toxin has been estimated to be approximately 2–3 ng/kg, making it second only to botulinum toxin as the deadliest toxin in the world.

The bacterium synthesizes the tetanus toxin as a 150 kDa polypeptide chain, which is composed of 2 subunits: A (50 kDa), and B (100 kDa). Similar to botulin toxin, it also has zinc-dependent endopeptidases with similar physical and chemical properties. The B-chain binds to disialogangliosides on the neuronal membrane and contains a translocation domain which aids the movement of the protein across that membrane and into the neuron, while the A-chain cleaves the synaptobrevin. This protein is necessary for vesicle fusion to membranes. Cleavage of the synaptobrevin inhibits neurotransmitter exocytosis in the inhibitory interneurons. The effect of the toxin is to block the release of inhibitory neurotransmitters (glycine and gamma-amino butyric acid (GABA)) across the synaptic cleft, which is required in checking of the nervous impulse. Blocking the release of GABA, which is mainly a neurotransmitter that inhibits motor neurons, causes a violent spastic paralysis which is a characteristic symptom of this toxin.

The routes of exposure are similar to those in botulin toxin, however tetanus toxin has a clinical picture of poisoning. The first symptom of poisoning is a slack jaw, followed by the so-called sardonic smile, stiffness of the neck, difficulty swallowing and muscle contractions in the torso and limbs, with a characteristic bent back (opisthotonos). Next, the cramps become stronger (even breaking the long bones and spine), and are very painful and exhausting.

Treatment is based on administration of anti-tetanus immunoglobulin (antitoxin) that bind the toxin while circulating in the blood. Adjuvant therapy consists of painkillers and relieving the cramps.

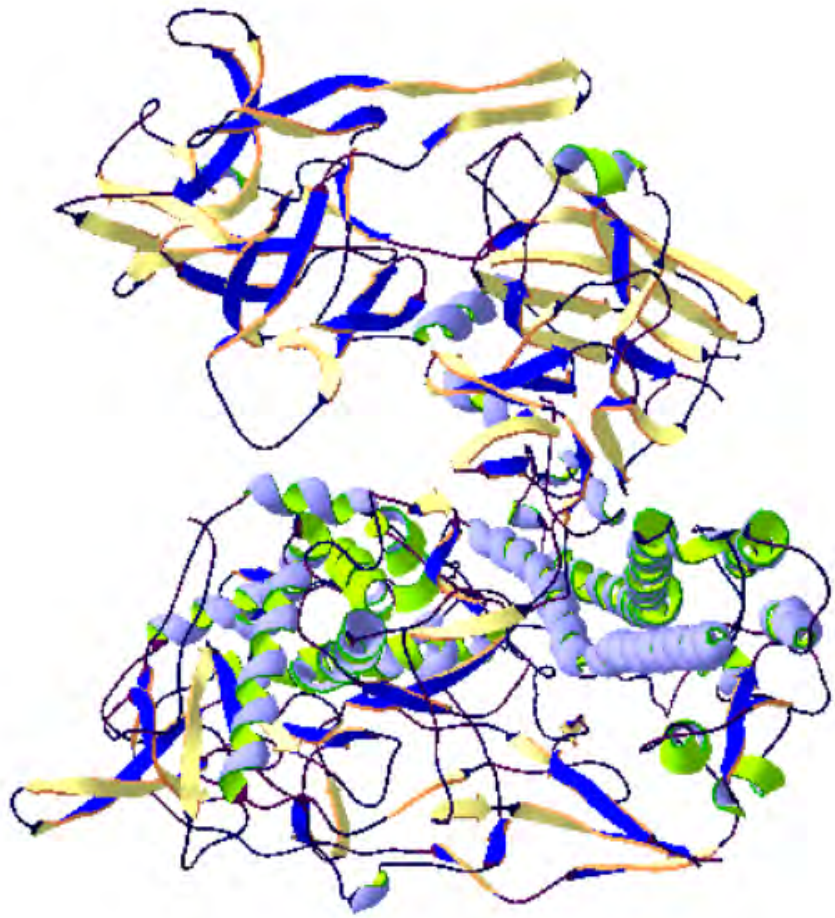

Figure 4. Tetanus toxin structure

4.1.3. Enterotoxins

Enterotoxins are a kind of exotoxin that is secreted by some pathogens. In nature, this group is mostly responsible for food poisoning. Enterotoxins include staphylococcal enterotoxins and AB5 group enterotoxins (i.e. cholera toxins), Shiga toxins and heat-labile enterotoxins produced by *Escherichia coli*.

Staphylococcal enterotoxins are a super antigen able to activate many T lymphocytes, causing overproduction of cytokines, which are responsible for inflammation. Enterotoxins from the AB5 group bind with protein G and cause the transport of sodium and water ions outside the cell. The LD_{50} at oral administration of staphylococcal enterotoxin is about 25 μg/kg body weight; the

LD_{50} of the Shiga toxin is 0.5–20 μg/kg body weight, and the LD_{50} of cholera toxin is 260 μg/kg body weight.

Staphylococcus aureus, which is responsible for toxin production, is a very rapidly spreading bacterium. Usually, the bacteria are present in the throat, nasal cavity, and crotch/anus. There is no vaccine for staphylococcus, and the disease itself is resistant to antibiotics. *Staphylococcus aureus* is well developed in meat and dairy products. Its enterotoxins are mainly produced by *Staphylococcus* microorganisms, which are also present in food and cause food poisoning in humans. However, it is rare that they cause death. The symptoms persist for one or two days and mainly include abdominal pain, nausea, diarrhoea and vomiting. They contain 21 serotypes: A, B, C1, C2, C3, D, E, G, G2, H, I, J, K, L, M, N, O, P, Q, R, S, T, U, U2 and V. Serotypes A, B, C1, C2, C3, D and E are known as 'classic enterotoxins', while the others are 'new types'. Staphylococcal enterotoxins are proteins with a mass of 26–35 kDa, having single polypeptide chains. These proteins are highly soluble in both water and salt solutions. Staphylococcal enterotoxins are highly resistant to proteolytic enzymes (pepsin, trypsin), exhibiting unchanged biological activity in the gastrointestinal tract. These toxins are also resistant to gamma radiation, a wide pH range ($2 < pH < 12$), and dehydration. High thermal stability is characteristic of staphylococcal enterotoxins, which makes them potential food poisoning agents.

As superantygens they have a non-specific way of binding with Class II TCR complex MHC molecules. Consequently, T lymphocytes CD4 and CD8 are activated and overproduction of cytokines occurs. T lymphocytes are subjected to apoptosis or enter a state of anergy. Natural food poisoning is usually caused by A and D staphylococcal enterotoxins, but less frequently also by the B and C types.

The most dangerous enterotoxin, with great potential for use in bioterrorism, is Staphylococcal Enterotoxin B (SEB). It is the most heat resistant toxin, and is detected even in samples of thermally sterilized food. Enterotoxin B induces toxic shock syndrome in the body, which is a very strong immune response by the human organism. Strong food poisoning occurring very quickly can lead to dehydration and death. In the case of SEB being ingested into the body other than through food, for example by inhalation, it can trigger a septic response throughout the organism. The toxicity parameters for this type of poisoning are LD_{50} 20 μg/kg and ED_{50} 400 ng/kg.

Treatment is mainly based on conservative treatment, while inhalation intoxication is treated through the administration of anti-inflammatory steroids.

Enterotoxins in the AB5 group include Shiga toxin, heat-labile enterotoxin and cholera toxin. They have a similar construction and consist of a heterodimeric subunit A, containing polypeptide chains A1 and A2 linked by a disulphide bridge, and a homopentametric subunit B. Subunit A2 is linked with subunits A1 and B. Subunit B is built of five monomers. They are arranged in a ring that contains binding spots for the cellular membrane receptors.

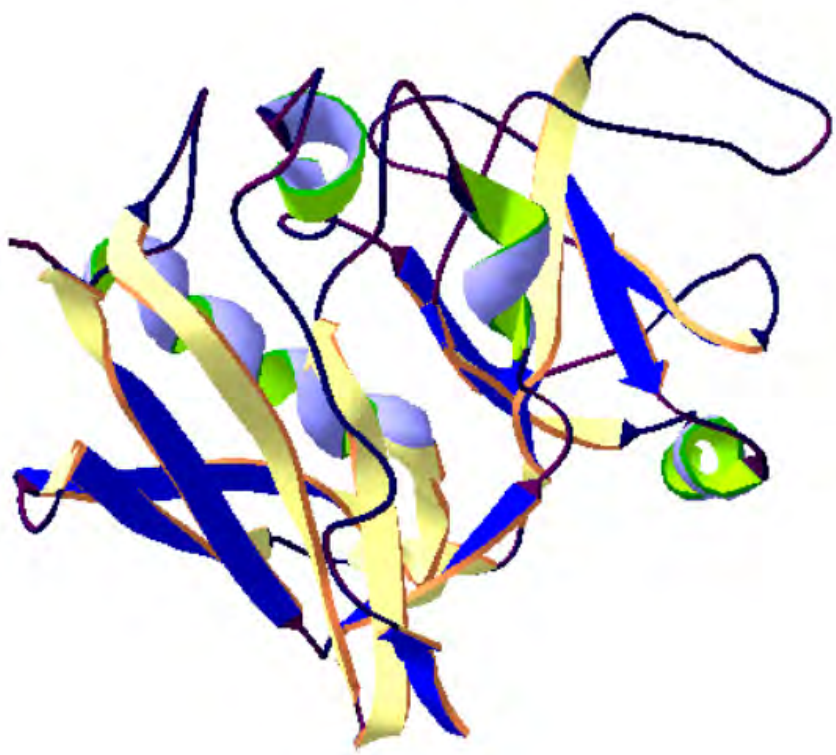

Figure 6. Enterotoxin type B structure

Shiga toxins are produced by *Shigella dysenteriae*. The Shiga family also includes toxins produced by *Escherichia coli* (O157:H7). A Shiga toxin contains a subunit A1 with a mass of 27.5 kDA, A2 with a mass of 4.5 kDa, and B with a mass of 7.7 kDa. These toxins are responsible for inhibition of protein synthesis in sensitive eukaryotic cells. Protein synthesis is blocked by the removal of adenine residue from the 28S rRNA of the 60S ribosome. Shiga toxin-mediated damage to the ribosome induces a response in cells called ribotoxic stress response, which is both pro-inflammatory and pro-apoptotic. The first symptoms occur after 6 hours of poisoning. The only viable medical intervention is supportive treatment.

4.1.4. Clostridium perfringens toxins

Clostridium perfringens is a rod-shaped, anaerobic, Gram-positive, spore-forming bacteria common in many different microbiota, and are found in the soil, marine sediment, in decaying vegetation, and in the intestinal tract of humans and other animals. This bacteria is able to produce at least 17 different

toxins. However four of them (alpha (CPA), beta (CPB), epsilon (ETX) and iota (ITX)), are the major toxins and have high toxic potential. Alpha and epsilon toxins have been classified by the CDC as Category B bioterrorism agents, which suggests their potential in these kinds of acts.

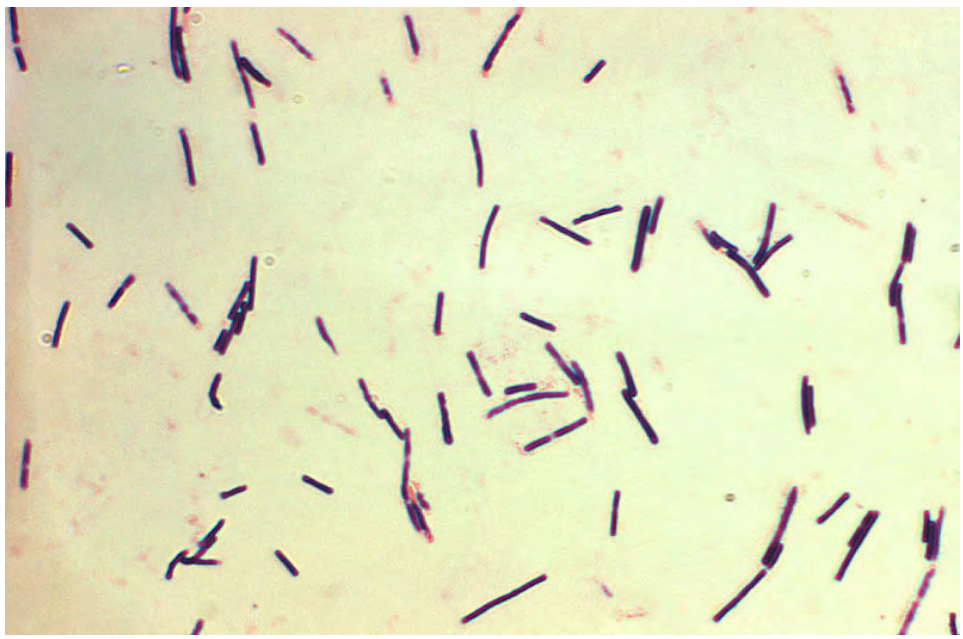

Figure 7. *Clostridium perfringens* (*figure used with permission under Creative Commons license*)

CPA is a 43 kDa protein containing two domains, an alpha-helical N-terminal domain harbouring the phospholipase C active site, and an alpha-sandwich C-terminal domain involved in membrane binding. This toxin is a classic example of a toxin that modifies cell membranes through enzymatic activity degrading phosphatidylcholine and sphingomyelin the components of the eukaryotic cell membranes. This damages the cell membrane which results in cell lysis. Additionally, the lipolysis of the cell membrane activates an arachidonic cascade resulting in the formation of thromboxanes, leukotrienes and prostaglandins, which activate the inflammation cascade and produce vasoconstriction. The ensuing intravascular haemolysis and capillary damage, platelet aggregation and hepatic necrosis results in multiple organ failure.

ETX is the third most potent biological toxin after botulinum toxin and tetanus toxin, with an LD_{50} of about 70–100 ng/kg. The active toxin is a protein with a molecular mass of 29 kDa, with relative resistance to proteases in the gastrointestinal tracts of mammals. This toxin is most stable at room temperature for up to a few weeks, and far longer at colder temperatures.

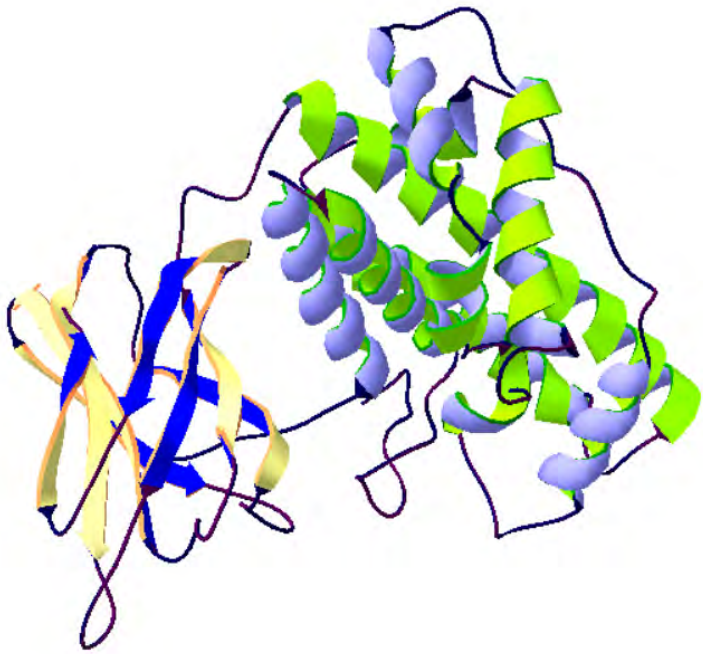

Figure 8. Alfa toxin structure

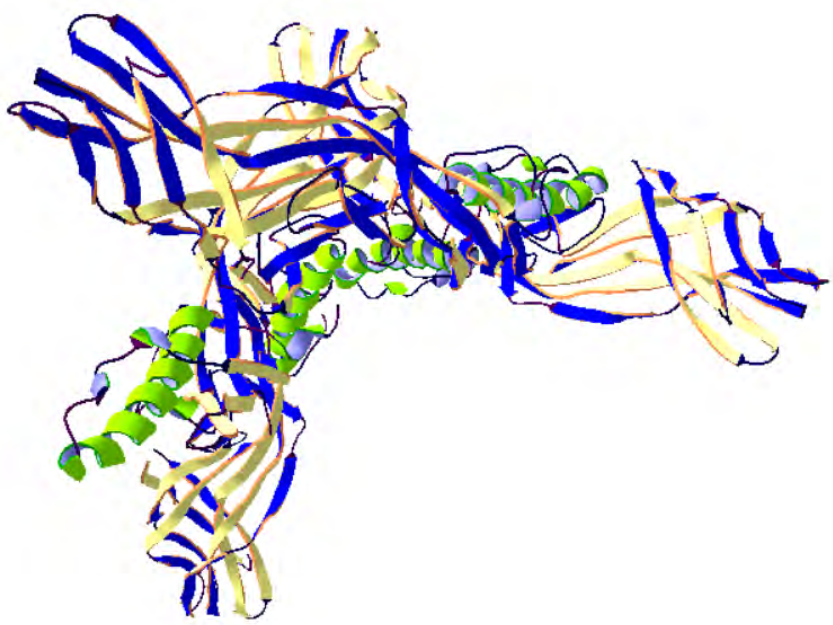

Figure 9. Epsilon toxin structure

Epsilon toxin interacts with the cellular membrane and creates pores within the membrane that modify its porousness and quickly becomes cytotoxic. Epsilon toxin induces pore formation in eukaryotic cell membranes *via* detergent-resistant, and cholesterol-rich membrane domains (lipid rafts). This damage results in very fast degenerative and necrotic changes in cells, leading to organ failure. The greatest potential for this toxin's use in bioterrorism is in an aerosolized form that can be used as a bioterrorist weapon. Additionally, this toxin can be dispersed in food intended for human consumption.

4.2. Plant toxins

Plant toxins are substances produced by plants as part of their defence mechanism. Pests and herbivores do not differentiate between particular plants. Plant toxins are additionally produced to protect the plants against various other threats, such as bacteria, fungi and insects. They are usually proteins or secondary metabolites which are not essential to the life of the plant producing them. Poisonous plants have a seed, root, leaf, stalk, fruit or juice from which even a relatively small amount, either received unwittingly or administered deliberately, can harm the human organism. In some plants, the poisonous constituents occur throughout the whole plant. In others, they are present in one or more parts. Toxins are produced by plants from every climate zone and in almost every ecosystem. Their easy accessibility has allowed them to kill people since ancient times. Human ingenuity in this area was unlimited. The pathways of plant toxins into the body can be very different, but most often are *via* inhalation or food. Also, the impact spectrum is very extensive and covers, amongst others, inhibition of the activity of cellular enzymes, actions on cell receptors (activation or inhibition), and interference with nucleic acids. The main chemical types of biological toxins are alkaloids, glycosides, tannins and lectins (a type of protein).

4.2.1. Ricin

Ricin is a toxin of plant origin, obtained from whole seeds or the waste of the castorbean plant (*Ricinus communis*), from which castor oil is pressed. The plant is endemic in eastern Africa and Asia, although nowadays it also grows in regions of subtropical and temperate climate. The yield from 1 kg of seeds is about 1 gram of pure ricin. Pure, extracted ricin is a white and yellow powder that is stable in the environment.

Ricin belongs to a group of proteins called lectins, which are proteins with high affinity to two glycoprotein chains: A and B, linked by a disulphide bridge. Chain B is a lectin, which binds with glycoprotein containing the mannose that occurs in the outer layer of the cellular membrane, facilitating endocytosis

of the toxin to the cell cytosol. Chain A has active RNA N-glycosidase, which causes decomposition of the glycoside bond in the adenine nucleotides of RNA molecules present in the large (60S) and small (28S) ribosome subunits. Chain A causes inactivation of ribosomes in the cells and blocks protein synthesis, which results in cellular death. One ricin molecule is able to deactivate as many as 2,000 ribosomes within 1 minute.

Figure 10. *Ricinus communis* in nature

Ricin's toxicity depends on the exposure route and dose. The LD_{50} value for humans after ingestion is estimated at about 22–25 μg/kg body weight. Just 5–6 grains of castorbean plant seeds are considered a lethal dose to children, while for adults it is about 20 grains. A lethal dose following inhalation is 3 μg/kg body weight. It is estimated that if 8 tons of ricin are released as aerosol in an area of 100 km^2, 50% of the population in the area would die.

Following ingestion of castorbean plant seeds or food contaminated with the toxin, the alimentary tract is the first to be affected and damaged. The symptoms do not occur immediately upon ingestion but after a few days of vomiting and diarrhoea, as a result of the alimentary tract irritation. This causes bodily dehydration, which is followed by alimentary tract bleeding and necrosis of the liver, kidneys and pancreas. Vascular collapse tends to follow next. If the toxin enters *via* the respiratory tract, the symptoms that occur within several hours following inhalation include

fever, coughing and progressive respiratory insufficiency. In more severe cases, pulmonary oedema, hypotension and vascular collapse are observed. Intramuscular administration of the toxin causes oedema in the injection area and necrosis of local lymph nodes, while other typical symptoms include gastrointestinal bleeding and renal necrosis. Death as a result of ricin poisoning occurs, on average, within 36–48 hours, regardless of the route of exposure. If the patient does not die within 3–5 days, their chance of being cured and surviving is high.

Figure 11. *Ricinus communis* seeds (*figure used with permission under Creative Commons license*)

Victims of ricin poisoning are not dangerous to their environment. However, there are no detailed treatment methods or vaccines available yet. Even so, experimental vaccines are in development, and tests on animals have proven their effectiveness.

Considering its high toxicity and the easy separation of the toxin from *Ricinus communis* plant seeds, there is a high risk of ricin use in bioterrorist attacks. Considering the various advantages of castor oil's use in cosmetics and its low production costs, 1.5 million tons of castor oil are produced in 30 countries

around the world, which creates the opportunity to obtain large amounts of ricin toxin. A small toxic dose and many potential exposure routes also make it a very good biological agent for use in bioterrorism.

Figure 12. Ricin structure

The identification of an aerosol attack can be based on observation of lung symptoms. Lung oedema occurs much later (1–3 days after exposure) than with staphylococcus enterotoxin (about 12h), or phosgene (about 6h). Additionally, the symptoms will deepen despite antibiotic therapy, contrary to their effect on infectious agents. Patients with acute respiratory poisoning require intensive ARDS therapy: oxygen, intubation, artificial ventilation, and haemodynamic monitoring. In gastrointestinal poisoning gastric lavage should be performed, followed by laxatives. Activated carbon is not effective against ricin, which has a high molecular weight. Instead, as supportive treatment proper hydration and electrolyte status of the patient is required.

4.2.2. Abrin

Similarly to ricin, abrin is also a toxin of plant origin obtained from *Abrus precatorius*, a plant also known as the 'rosary pea'. Originally, it used to grow in South-East Asia and in Guinea, Africa, but nowadays can be found in many tropical regions all over the world. The structure of abrin is similar to the

structure of ricin toxin. Abrin consists of two polypeptide chains: A and B, linked by a disulphide bridge. Chain B, which has the properties of lectin, plays the role of a binding domain and is supposed to connect with glycoprotein receptors on cell surfaces. Chain A is RNA N-glycosidase, as in ricin. The mechanism of abrin action involves blocking of the translation in the cells into which it has penetrated. In the cell cytosol, chain A separates the C-N bond in adenines located in a small subunit of 28S rRNA ribosome. Adenine depurination destabilises rRNA, and so consequently the synthesis of proteins is inhibited.

Figure 13. *Abrus precatorius* (*figure used with permission under Creative Commons license*)

Abrin is 30 times more toxic than ricin, with an LD_{50} estimated at about 0.7 μg/kg body weight. Additionally, all parts of the *Abrus precatorius* plant are toxic but the highest concentration of the toxin occurs in the seeds. The pure, extracted form of abrin is a white and yellow powder that is stable in the environment. It can also be used in several other forms such as a mist, or dissolved in water.

A lethal dose following ingestion of the toxin amounts to 0.1–1 μg/kg body weight, which means that swallowing just 1-2 grains of rosary peas can cause death. The toxin can penetrate into the body by ingestion of the seeds, through the respiratory tract or by injection.

Figure 14. *Abrus precatorius* seeds (*figure used with permission under Creative Commons license*)

Poisoning with abrin by ingestion of seeds or food infected with the toxin causes severe abdominal pain, vomiting and diarrhoea. Renal insufficiency then develops. In most cases, bleeding from the alimentary tract is also observed. If abrin is absorbed by inhalation, the symptoms include pulmonary oedema, hypertension in the pulmonary arteries and haemolysis of erythrocytes. Death usually occurs 36–72 hours after exposure, depending on the route of exposure and dose. It is considered that if the patient does not die after 3–5 days, there is a chance that they will survive.

Because of their attractive appearance, the seeds of *Abrus precatorius* are used as beads for rosaries and bracelets, which causes more people to be exposed to the toxic action of abrin. Since there are no available drugs or vaccines against abrin, treatment is supportive and based on minimising the effects of the poisoning. The type of medical care administered depends on several factors, with the infection route being the most important, and includes support for the respiratory tract, intravenous administration of fluids and stabilisation of blood pressure. Possible neurological symptoms after exposure include hallucinations, reduced consciousness and convulsions.

Recognition of the poisoning as well as its medical treatment are identical to the response to ricin poisoning.

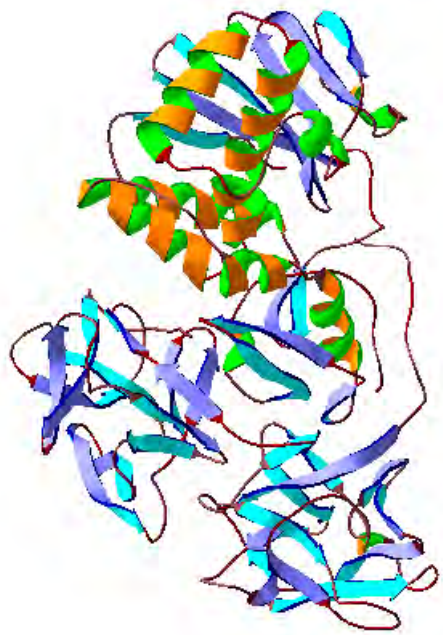

Figure 15. Abrin structure

Abrin is the strongest plant toxin, which means it can be potentially used as a biological weapon. Because only a small dose is required to cause severe poisoning, and because of the possibility of using abrin in several forms as well as its fast action, make it attractive to bioterrorists. The common occurrence of *Abrus precatorius* in tropical regions, which makes access to it quick and easy, is another advantage.

4.2.3. Aconitine

Aconitine, also known as the 'Queen of Poisons', is a plant toxin present in the very commonly-occurring *Aconitum napellus Rchb*. This is a species of plant belonging to the glaucoma family. It occurs naturally in the temperate climate zone of Eurasia. The name Aconitum may have been derived from the Greek word 'akone', meaning 'rocky' or 'ravine', which is where the plant usually grows. The flowers of Aconitum are considered to be very beautiful (with a medium to dark semi-saturated blue-purple colour), which means that this plant is often cultured in gardens as a decorative flower.

Aconitine is a secondary metabolite of the Aconitum plant and chemically is classified as a norditerpenoid alkaloid. Aconitine is a C19-norditerpenoid, barely

soluble in water, but very soluble in organic solvents such as chloroform or diethyl ether. Aconitine is also soluble in mixtures of alcohol and water if the concentration of alcohol is high enough. In nature it occurs mainly in the leaves, stems and roots of the Aconitum plant. The LD parameter is in the range 0.1–1 mg/kg of body mass. This toxin is very well absorbed by mucous membranes and skin.

Figure 16. *Aconitum napellus* in nature

The toxic action of aconitine results from its interaction with the voltage-dependent sodium-ion channels, which are proteins in the cell membranes of excitable tissues, such as cardiac and skeletal muscles and neurons. Normally, the sodium channels close very rapidly, but depolarization of the membrane potential causes the opening (activation) of potassium channels and potassium efflux, which results in repolarization of the membrane potential. Binding of aconite increase of the permeability of the membrane for sodium ions, resulting in a huge sodium influx to the cell. As a result, the membrane rapidly depolarizes. Due to the strong depolarization, the permeability of the membrane to potassium ions increases very quickly, resulting in a potassium reflux that releases the positive charge from the cell. These events result in the transmission of action potentials being suppressed, leading to non-excitable target cells or paralysis.

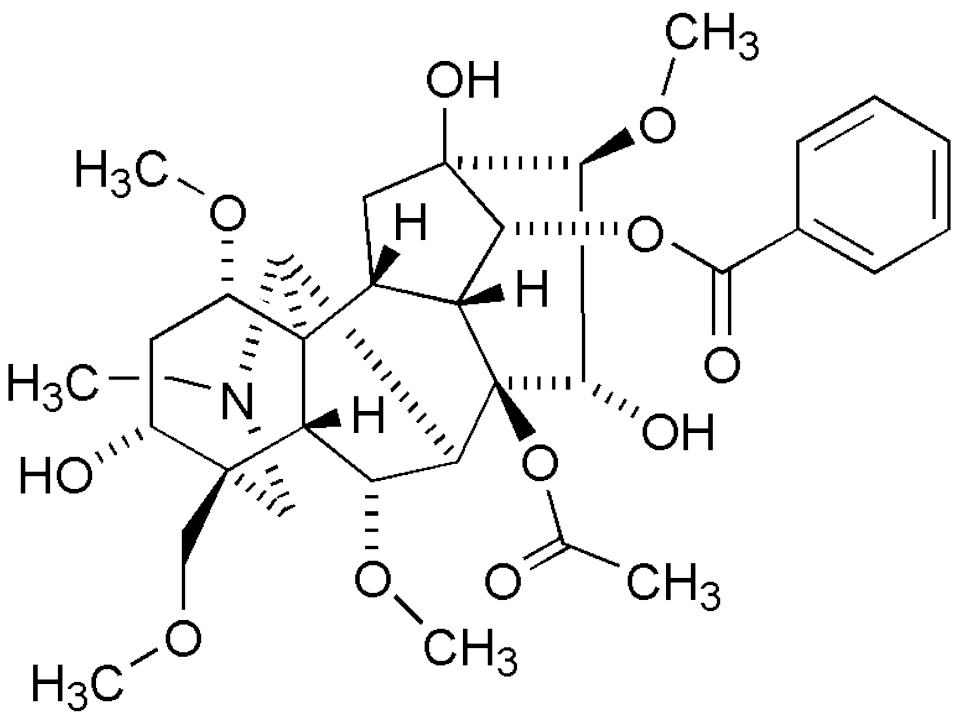

Figure 17. Aconitine chemical structure

Depending on the route of exposure, observable toxic effects include localised numbness, diarrhoea, convulsions, arrhythmia and death. After administration, aconitine initially stimulates all vegetative parasympathetic centres and subcortical nuclei, causing cardiac failure. This leads to severe vomiting, colicky diarrhoea, intense pain and then paralysis of the skeletal muscles. Cardiac failure is a cascade process beginning with cardiac arrest (irritation of the vagus nerve), followed by sinus node stimulation and accelerated cardiac function. Next comes increased sensitivity of autonomic heart ligaments, leading to hypersensitivity, dissociation of heart movements, cardiac arrhythmias, ventricular pacemakers, atrial and cardiac tremor, and cardiac arrest in diastole. Death is caused by paralysis of the centres of the extended core. Respiratory failure and cardiac arrest follow, all with full patient awareness.

The medical response should be first based on treatment with stimulatory substances – caffeine, adrenaline, amphetamine; antiarrhythmic drug should then be given (good results have been reported for lidocaine). Considering the fact that aconitine acts as an agonist of the sodium channel receptor, antiarrhythmic agents which block the sodium channel (Vaughan-Williams' Classification I) should be chosen for the acute therapy of aconitine induced arrhythmia.

4.3. Fungal toxins

Toxin-forming fungi produce mycotoxins present in feed, cereals and food products. The toxins have a wide spectrum of action and are harmful to humans, animals, plants and microorganisms. They impair various metabolic processes as well as DNA replication and transcription. This type of toxin is very easy to produce, even in homemade labs, which increases the potential for their use in bioterrorist attacks.

4.3.1. T-2 toxin

T-2 toxin is a mycotoxin synthesised by different species of *Fusarium* fungi. Concentrations of the toxin that are potentially dangerous to health occur in mouldy cereal grains and other inappropriately-stored agricultural products. Of all studied grains, the ones most prone to being infected include oat, corn, wheat and rye. The toxin can be found all over the world, particularly in the tropical regions, as heat and high humidity foster development of the *Fusarium* fungi.

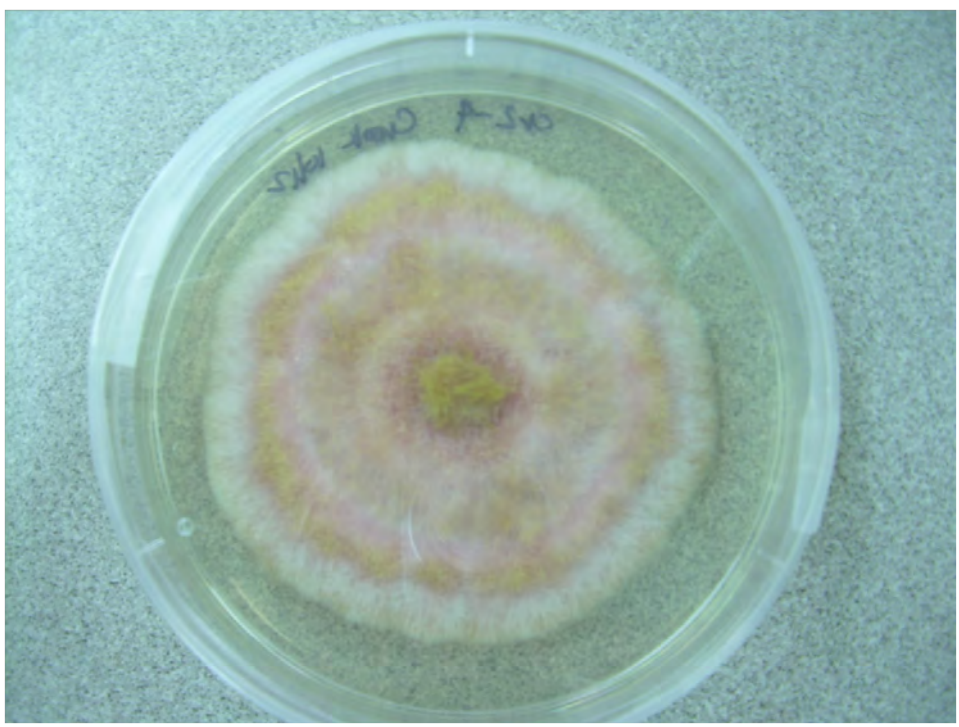

Figure 18. *Fusarium* fungi (*figure used with permission under Creative Commons license*)

From the chemical point of view the toxin is a low-molecular organic compound with a mass of 466 Da. The compound has a tetracyclic sesquiterpene 12, 13-epoxytrichotocenic cyclic arrangement and is part of the Trichothecenes group. This group includes various toxins such as neosolaniol, saratoxin, diacetoxyscirpenol, crocetin and HT-2. However, T-2 possesses the highest potential.

The T-2 toxin is very stable and resistant to high temperatures (it is not damaged during regular food preparation), and UV radiation. Its toxic action can be neutralised by heating at 200°C–210°C for 30 to 40 minutes, or by adding sodium hypochlorite. T-2 is non-soluble in water but is soluble in chloroform, acetone, ethanol and methanol. T-2 mycotoxin can penetrate into the body with food, or as a smoke or aerosol sprayed by different dispersive systems.

The action mechanism of the compound works thanks to the presence of an epoxy ring in its structure, which is capable of intracellular reactions with nucleophilic units. It reacts with DNA and RNA molecules by inhibiting their synthesis in the cell, and with membrane phospholipids by damaging cellular structures. Additionally, the interaction of T-2 with ribosomes and enzymes participating in translation has also been demonstrated. This toxin prevents formation of peptide bonds in the centre of peptidyl transferase located in the ribosome 60S subunit, which inhibits synthesis of eukaryotic proteins.

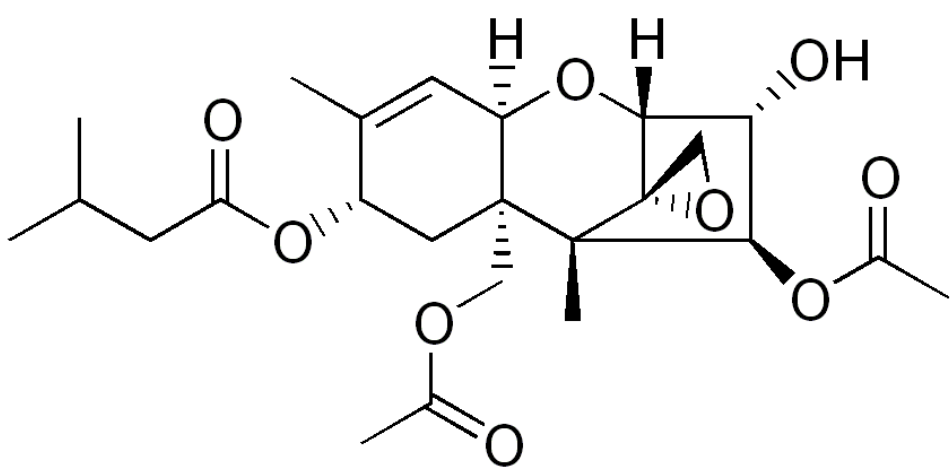

Figure 19. T-2 toxin chemical structure

The toxic action of T-2 causes apoptosis and nnecrosis of cells, immunosuppression and severe damage to skin tissue and other organs. Immunosuppression is an effect of high doses that damage the bone marrow, pancreas, thymus and lymph nodes, resulting in impeded functions of the immune system. Contrary to most biological toxins that do not affect the skin, T-2 is a strong skin irritant. Symptoms occur within

several minutes of exposure. Skin damage caused by the toxin is 400 times more severe than after using sulphur yperite, and include sintradermal haemorrhage and necrosis. This effect is observed even in nanograms of toxin.

Ingestion of the toxin results in abdominal pain after 15 minutes, up to one hour following ingestion. Moreover, irritation of the throat and severe diarrhoea occur.

Scientists have suggested 4 clinical stages for a bioterrorist attack using T-2 mycotoxin. The first stage would include inflammation of the alimentary tract mucous membrane, and progressive dehydration. After about 10 days, anaemia, thrombocytopenia and leukopenia would develop in the next stage. Petechiae and haemorrhages, followed by necrotic lesions in the alimentary tract or larynx leading to sepsis and death would occur in the third stage. The fourth stage would be the recovery of survivors.

At present there are no drugs counteracting poisoning with T-2 mycotoxin. Treatment involves maintaining the functions of the circulatory, respiratory and alimentary tract. These actions include administering oxygen and maintaining hydration. Polyethylene glycol is effective in removing the toxin from the skin's surface. Experiments carried out on mice revealed that T-2 toxin is strongly adsorbed by active carbon, which suggests that the same results can be obtained for humans.

T-2 toxin, considering the lack of accurate data on its action mechanisms in the human body, could become a perfect B weapon. Moreover, studies of T-2 have not gone beyond the laboratory level and information on its lethal doses or effects are estimates and based on animal reactions. Another advantage of the toxin in biological warfare is the lack of drugs counteracting it, which greatly delays or prevents recovery. Additionally, this toxin was used in biological warfare in Iraq (by Saddam Husain), as well as by Soviet forces in Kampuchea and Afghanistan (1979–81). There were also reports of "yellow rain" in Laos during the Vietnam war.

4.3.2. Aflatoxin

Aflatoxin produced by *Aspergillus flavus* and *Aspergillus parasiticus* is one of the most common fungal toxins. There are 6 identified metabolites: B1, B2, G1, G2, M1 and M2. The LD_{50} for the toxin following oral administration is 0.003–18 mg/kg body weight. They all contain a lactone ring which is bound with a benzene ring and two furan rings. The most potent toxin of this group is aflatoxin B1.

B1 aflatoxin is oxidised with P450, taking an epoxy form in the liver. A covalent bond is then formed with guanine N-7 nitrogen. A double bond in the extreme furan ring enables tight connection of aflatoxin with DNA. The toxin causes transversions of GC alkalis into TA in the p53 protein codon. It damages the mechanism responsible for DNA repair and impairs apoptosis. Aflatoxins also affect cellular respiration paths, post-translation modification of proteins and methylation of nucleic acids and proteins, which lead to the formation of

neoplasms. For this reason, the aflatoxin B1 is a genotoxic hepatocarcinogen with its exposure strongly linked to the development of hepatocellular carcinoma. The oral LD_{50} range of aflatoxin B1 is estimated to be 0.3–17.9 mg/kg body weight for most animal species. Symptoms of acute poisoning include anorexia, malaise, low-grade fever as well acute necrosis of liver.

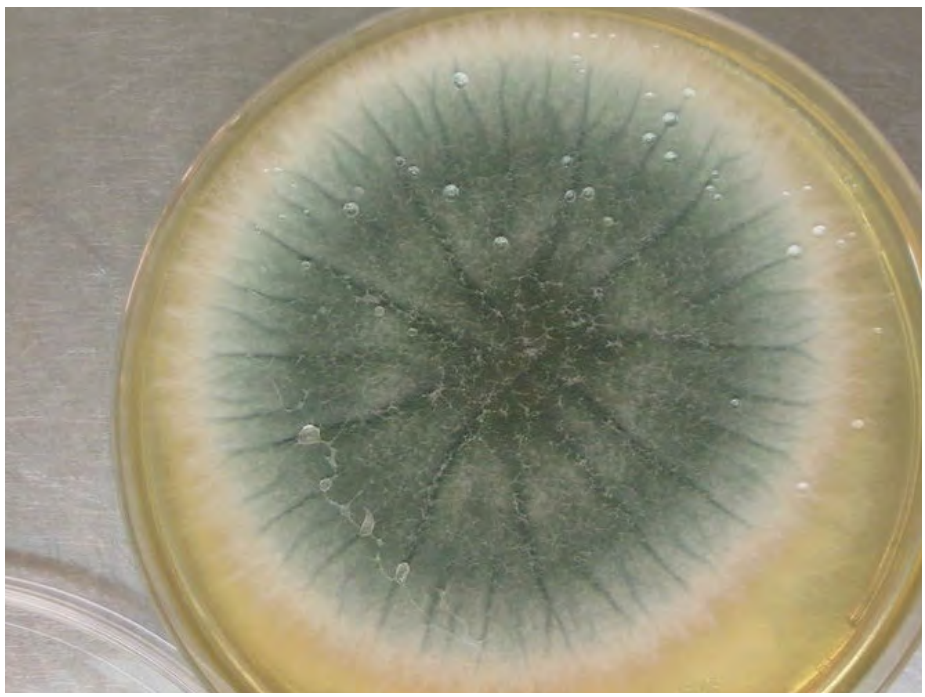

Figure 20. *Aspergillus sp* (*figure used with permission under Creative Commons license*)

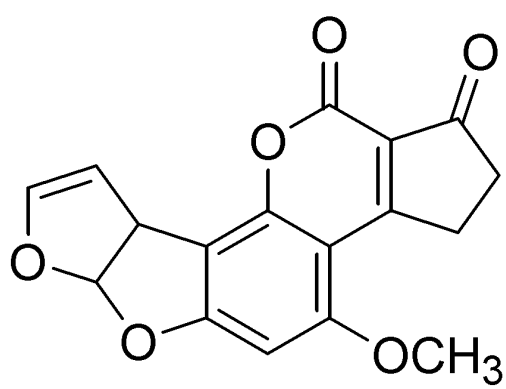

Figure 21. B1 aflatoxin chemical structure

4.3.3. Ochratoxin A

This toxin, produced by *Aspergillus ochraceus*, is a peptide of L-phenylalanine amino acid bound with iso coumaric acid *via* an amine group. Ochratoxin A contains a chlorine atom in the benzene ring. The LD_{50} dose for ochratoxin administered orally is 20–46 mg/kg body weight. Ochratoxin A gets into the alimentary tract. It binds with albumins of high affinity. Ochratoxin A is linked with IIA or IIIA subdomains on the human serum albumin. Organic anions transporting peptides (OAPTs) are located in the liver, while an organic anion transporter (OAT) can be found in the kidneys. These are molecular structures, whose active function is cellular capturing of ochratoxin A. Ochratoxin A is a protein synthesis inhibitor that inhibits the activity of the t-RNA synthase enzymes phenylalanine and phenylalanine hydroxylase. It reduces ATP production in the cell by impeding the activity of phosphoenolpyruvate kinase. Moreover, the toxin can bind with proteins in the mitochondria, inhibiting transport of electrons and blocking phosphorane transport. Similarly to aflatoxin, it has genotoxic properties. The toxin metabolites can bind with DNA causing mutations, and consequently neoplasms. During metabolic transformations of the toxin, overproduction of free radicals is observed, which causes oxidative damage to DNA, induces apoptosis, impairs mitosis and contributes to the instability of chromosomes. It also has a negative impact on the cellular cycle.

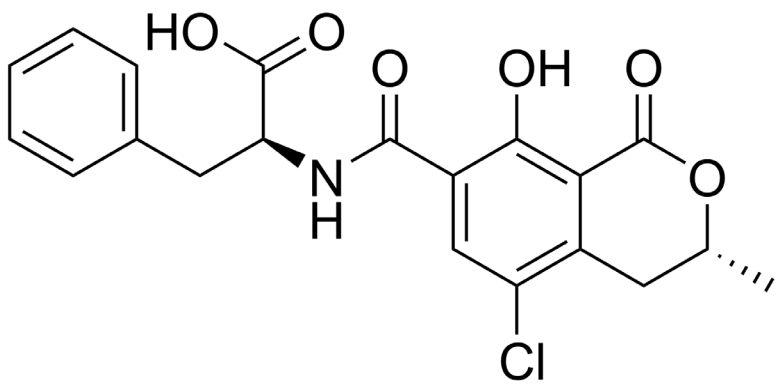

Figure 22. Ochratoxin A chemical structure

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