ANNEX 2: TOXINS

2.1 WHAT IS A TOXIN?

'Toxin' has no commonly accepted meaning within the scientific literature. Some writers use the term for any toxic substance. Some reserve it for proteinaceous antigens. Some differentiate between 'chemical toxins' and 'biological toxins'. Some write of 'true toxins'. There is confusion.

The confusion may be of little account to the health authorities of Member States unless they become obliged to seek international assistance in the event of toxin-warfare attack, actual or threatened. It may then be important to understand how toxins are treated in the Biological and Chemical Weapons Conventions, which are potential sources of such assistance.

The 1972 Biological and Toxin Weapons Convention extends to "toxins whatever their origin or method of production". It does not say what it takes toxins to be, but its travaux préparatoires show that the term is intended to mean toxic chemicals produced by living organisms. Prominent here are the actions of the United States. On 14 February 1970, during the negotiation of the Convention, the United States announced that it had decided to renounce offensive preparations for the use of toxins as a method of warfare. Shortly afterwards, it informed the treaty-negotiating body that toxins "are poisonous substances produced by biological organisms, including microbes, animals, and plants" (A2.01); and it has since reiterated, even expanded, that understanding in the legislation implementing the Convention in US law. This legislation states that "the term 'toxin' means the toxic material of plants, animals, microorganisms, viruses, fungi, or infectious substances, or a recombinant molecule, whatever its origin or method of production, including -- (A) any poisonous substance or biological product that may be engineered as a result of biotechnology produced by a living organism; or (B) any poisonous isomer or biological product, homolog, or derivative of such a substance" (A2.02). The essence of this definition evidently found favour with all other states parties to the Convention, for the Final Declaration from the Second Biological Weapons Convention Review Conference states that "toxins (both proteinaceous and non-proteinaceous) of a microbial, animal or vegetable nature and their synthetically produced analogues are covered" by the treaty (A2.03).

Inasmuch as toxins are both toxic and chemical in nature, they automatically fall within the scope of the 1993 Chemical Weapons Convention, which states that "toxic chemical" means: “Any
chemical which through its chemical action on life processes can
cause death, temporary incapacitation or permanent harm to humans
or animals. This includes all such chemicals, regardless of their origin
or of their method of production, and regardless of whether they are
produced in facilities, in munitions or elsewhere."

So, although there is no consensus on the term among
scientists, international law regards a wide range of substances as
‘toxins’. At one end of the range are the bacterial toxins, such as
botulin and staphylococcal enterotoxin, both of which have in
the past been stockpiled for weapons purposes. They are high-
molecular-weight proteins that can at present be produced on a
significant scale only by processes of industrial microbiology. In the
middle of the range are the snake poisons, insect venoms, plant
alkaloids and a host of other such substances, some of which are
becoming accessible to chemical synthesis and some of which, for
example curare, batrachotoxin and ricin, have been used as weapons.
At the other end of the range are small molecules such as potassium
fluoroacetate (found in the plant *Dicephalatum cymosum*) that are
typically synthesized by chemical process when they are needed even
though they are also produced by certain living organisms, thereby
falling within the legal definition of ‘toxin’. Hydrogen cyanide is
another such toxin. It occurs in some 400 varieties of plant, in certain
animals, and is synthesized by at least one bacterium (*Bacillus
pyocyaneus*).

In the sense of the Biological and Toxin Weapons Convention,'
toxin’ includes substances for which scientists would not normally use
the term. For example, there are chemicals that occur naturally in the
human body which would have toxic effects if administered in large
enough quantity. Where a scientist might observe a bioregulator, say,
the treaty would see a poisonous substance produced by a living
organism, in other words a toxin. Nor is this unreasonable. Wasp
venom, for example, is clearly a toxin, yet its active principle is
histamine, a human bioregulator. Although histamine might not itself
be made into an effective weapon, the same cannot necessarily be
said for other bioregulators.

Indeed, now that large-scale production processes for
biologically active peptides and similar substances are undergoing
rapid commercial development, bioregulators and other toxins
constitute a field rich in potential for weapons as well as for
pharmaceuticals, in particular weapons of intense disabling or
incapacitating power. So it is fortunate that this advance in
biotechnology should have coincided with the expanding
implementation of the Chemical Weapons Convention. For this is a
treaty that places its states parties under express obligation to ensure
that bioregulators and other toxins, like all other toxic chemicals, are
used only for the purposes that the treaty does not prohibit.
Described below are some of the toxins that have been weaponized in the past. Others, such as hydrogen cyanide and its derivative cyanogen chloride, are taken up in the annex on chemical agents (Annex 3). So also is a toxin that is finding widespread use as a riot control agent, oleoresin capsicum, also known as Agent OC.

2.2 BACTERIAL TOXINS

2.2.1 Staphylococcal enterotoxins

These toxins are a common cause of diarrhetic food-poisoning after ingestion of improperly handled food. They are proteins that range in size from 23 to 29 kilodaltons. They are thought to work by stimulating massive release of a variety of cytokines that then mediate the different toxic effects. The toxins are known in at least five antigenically distinct forms. Type B is the most studied. It is heat stable and, in aqueous solution, can withstand boiling. It is active by inhalation, by which route it causes a clinical syndrome markedly different, and often more disabling, than when ingested. It has been studied as a warfare agent of the incapacitating type. The median disabling dose for human beings by inhalation has been estimated at 0.0004 µg per kilogram bodyweight. The corresponding lethal dose is estimated as being 50 times larger. (A2.04)

Main clinical features

When *Staphylococcus aureus* contaminates food products and the resulting pre-formed toxin is ingested, symptoms, which are usually nausea, vomiting and diarrhoea, occur within 1-6 hours of eating the contaminated food.

After inhalation of staphylococcal enterotoxin B (SEB), intoxication becomes manifest within 3-12 hours, as through the sudden onset of fever, headache, chills, myalgias and a non-productive cough. More severe cases may develop dyspnoea and retrosternal chest pain. If toxin is swallowed, nausea, vomiting and diarrhoea will occur in many patients, and fluid losses may be substantial. The fever, with variable degrees of chills and prostration, may last up to five days, and the cough may persist for as long as four weeks.

Diagnosis and detection

The diagnosis of inhalation SEB intoxication is clinical and epidemiological. Patient samples are unlikely to test positive for the toxin following aerosol exposure unless the exposure is large and samples are obtained rapidly. Enterotoxins may be detected in environmental samples using a variety of antibody based tests.

Medical management

Supportive therapy has proved adequate in cases of accidental respiratory exposure to SEB aerosol. Hydration
and oxygenation will require close attention. In severe cases, where pulmonary oedema develops, ventilation with positive and expiratory pressure and diuretics may be necessary. Most patients would be expected to do well after the initial acute phase of their illness, but would remain unfit for normal activities for 1-2 weeks. (A2.05) The illness being an intoxication, no isolation or other quarantine measures are required.

Prophylaxis

There is no human vaccine available although several are in development, including ones that, in animal studies, have been shown to protect against inhalation exposure to SEB. Passive protection has also been demonstrated.

Stability/neutralization

SEB can be detoxified by treatment with 0.5 percent hypochlorite for 10-15 minutes.

2.2.2 Botulinal neurotoxins

These toxins are the cause of deadly food-poisoning from canned foodstuffs that have been improperly prepared. They are proteins of around 150 kilodaltons in size, and in culture are associated with other proteins to form complexes of some 300 – 900 kilodaltons. There are seven antigenically distinct forms of botulinal neurotoxin. Each consists of two chains, the heavier of which binds to cholinergic synapses. The internalized lighter chain is a zinc protease and acts by cleaving proteins involved in the process of acetylcholine release. Particular substrate specificity varies between the different serotypes and may correlate with observable differences in speed of onset of botulism and duration of paralysis. Botulinal toxins are the most acutely lethal of all toxic natural substances. In dry powder, the toxins may be stable for long periods. They are active by inhalation as well as ingestion, the clinical picture being much the same by either route. The type A toxin, particularly though not exclusively, has been studied as a warfare agent of the lethal type. Its median lethal dose for human beings by inhalation has recently been estimated at 0.003 µg per kilogram bodyweight. By ingestion the dose is estimated to be some three times smaller. (A2.04)

Main clinical features

Botulism, in the natural form caused by ingestion of bad food, is a dramatic disease that is frequently fatal for animals and human beings alike, a 60 percent mortality occurring in reported cases prior to 1950. It is well described in the medical literature. Inhalation botulism, on the other hand, is rare, but efforts have recently been made to describe it systematically (A2.04, A2.05, A2.06).
Following inhalation exposure, the onset of symptoms may begin within 1-3 days, the smaller the dose the longer the onset-time. At the start, bulbar palsies may be prominent with eye symptoms such as blurred vision due to mydriasis, diplopia, ptosis and photophobia, as well as other bulbar signs such as dysarthria, dysphonia and dysphagia. Skeletal-muscle paralysis follows, with symmetrical, descending and progressive weakness. This may culminate abruptly in respiratory failure.

**Diagnosis and detection**

Misdiagnosis of botulism is frequent (A2.06). Diagnosis depends upon identifying the presence of toxin from blood samples using some form of antigen/antibody reaction. In the natural disease, the bacterium and/or preformed toxin may be identified in unconsumed food samples.

**Medical management**

Treatment involves administration of immune globulin (either human or despeciated equine) to neutralize toxin which has not already bound to cholinergic synapses. This is coupled with supportive therapy. The most serious complication, and the most common cause of death in botulism, is respiratory failure secondary to paralysis of respiratory muscles. Intubation and ventilatory assistance will be needed, and tracheostomy may be required.

There is no infection and thus no requirement for isolation or special hygiene measures.

**Prophylaxis**

Toxoid vaccines against types A-F have been produced and evaluated in animal and human studies. Type A toxoid has a UK product license. The present toxoid vaccines require several doses over a period of weeks to produce protection. Primate studies have also demonstrated passive protection against inhalation or injection of toxin by equine or human immune globulin (A2.07). The level of protection is entirely dependent upon the stoichiometric relationship between the amount of circulating antibody versus the amount of toxin to which an individual may be exposed.

**Stability/neutralization**

Botulinal toxins are rather easily neutralized. In food or drink, heating to an internal temperature of 85°C for more than 5 minutes is sufficient. In the airborne state, the toxin is degraded by extremes of temperature or humidity. The decay-rate of aerosolized toxin has been estimated at 1-4
percent per minute, according to the weather conditions (A2.06). Contaminated surfaces should be cleaned with 0.1 percent hypochlorite solution if they cannot be avoided for the few hours to days that natural degradation would require.

2.3 FUNGAL TOXINS

Systematic attention to fungal toxins as important causes of illness lagged prior to the 1960s, so that the literature has developed mostly since the conclusion of the Biological and Toxin Weapons Convention. It is now well known that some fungi produce a single toxin, while others may produce many, and that different fungal genera may produce the same mycotoxin. Many genera, including Acremonium, Alternaria, Aspergillus, Claviceps, Fusarium and Penicillium produce mycotoxins. While the evidence indicates that ingestion of mouldy feed and fodder is the primary route leading to animal mycotoxicoses, airborne fungal spores and infested/infected plant particulates may also induce disease leading to death in animals and man. (A2.08) The weapons potential of such airborne activity has not been disregarded, although, in the 1992 returns of information under the BWC confidence-building measures in which Russia declared offensive biological research and development programmes during the period since 1946, it is stated: “In the opinion of the experts, mycotoxins have no military significance” (A2.09).

Nevertheless, two categories of mycotoxin have been discussed as warfare agents and should be noted briefly here: the aflatoxins and the trichothecenes. An aflatoxin has actually been weaponized, so it is known from reports by the UN Special Commission on Iraq. The synoptic UNSCOM report of January 1999 stated that the “question remains open regarding the aims and reasons of the choice of aflatoxin as an agent”, but went to state that one Iraqi document “refers to military requirements to produce liver cancer using aflatoxin and the efficacy against military and civilian targets” (A2.10). As for the trichothecenes, they were the subject of allegations of weapons use (“yellow rain”) in Cambodia and Laos during 1975-84 that have since been discredited (A2.11).

Aflatoxicosis in humans is associated with consumption of aflatoxin from food contaminated with the mould Aspergillus flavus. A number of aflatoxins ranging in potency (B1 > G1 > B2 > G2) are produced by Aspergillus, the relative proportions dependent on the species of mould. Jaundice, fever, ascites, oedema of the feet, and vomiting are symptoms associated with aflatoxicosis. In 397 patients estimated to have consumed 2-6 mg aflatoxin daily for a month, 106 fatalities occurred. Fatalities also followed estimated intakes of 12 mg kg⁻¹ aflatoxin B1. Five-year follow-up of survivors of acute poisoning (including liver biopsies) showed almost complete recovery. The
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Principal concern with aflatoxin (particularly B₁) is the possibility of liver cancer associated with chronic consumption of mouldy food.

Aflatoxin chemistry and metabolism are well described. Aflatoxin B₁ is metabolised to a range of metabolites by microsomal systems. The active metabolite is presumed to be aflatoxin B₁ 8-9 epoxide. Inactivation is dependent on glutathione conjugation, with susceptibility to acute intoxication dependent on activity of the enzyme glutathione-S-transferase. The AFB₁₂ epoxide binds covalently to a range of proteins which have both structural and enzymic functions. Protein phosphorylation is also altered by AFB₁₆. Aflatoxins are all genotoxic agents. (A2.12, A2.13)

Trichothecene mycotoxins are a group of structurally related toxins produced by the Fusarium fungi found on many crops, and also by other mould genera such as Stachybotrys. They are sesquiterpenoids of low molecular weight, in the range 250 – 550 dalton. Two of the better known toxins are T-2 and deoxynivalenol (or vomitoxin). Symptoms caused by the toxins are wide ranging and include vomiting, diarrhoea, ataxia and haemorrhaging. The toxins are immunosuppressants and inhibit protein synthesis at the ribosomal level. They bind to the 60S subunit of eukaryotic ribosomes altering peptidyl transferase activity. Inhibition of enzyme activity depends on toxin structure, and results in the failure either of polypeptide chain initiation or of its elongation. Toxicity of the toxins in in vitro test systems, varies by as much as four orders of magnitude. (A2.14)

In animals, the toxicity of T-2 is markedly species dependent. Vomiting is induced in cats at 0.1-0.2 mg kg⁻¹ after oral dosing. Guinea pigs are unaffected at 0.75 mg kg⁻¹ day⁻¹ in the diet, but develop irritation and ulceration of the gut at 2.5 mg kg⁻¹ day⁻¹. Immunosuppression is observed in rhesus monkeys at 0.5 mg kg⁻¹ and in mice at 20 mg kg⁻¹. LD₅₀ in mice following intraperitoneal administration is reported to be 5.2 mg kg⁻¹. (A2.15) So the toxicity of the trichothecenes in comparison with other toxins is relatively low. They are, however, unusual among toxins in their ability to damage the skin, causing skin pain, pruritis, vesicles, necrosis and sloughing of epidermis.

2.4 ALGAL AND OTHER PLANT TOXINS

2.4.1 Saxitoxin
Saxitoxin is one of the phycotoxins that contribute to paralytic shellfish poisoning. It can also, with difficulty, be synthesized. Consumption of seafood contaminated with marine algal toxins may cause either paralytic or diarrhetic shellfish poisoning (PSP or DSP). In addition to production by marine algae, PSP toxins can be made by certain
bacteria, cyanobacteria and red algae. PSP toxins, depending on substituent side groups, are small molecules of around 300 dalton. The parent compound, saxitoxin itself, is a powerful neurotoxin that binds with high affinity to sodium channels on cell membranes, inhibiting influx of sodium ions into cells without altering potassium ion efflux. Cell action potentials are suppressed, and paralysis results, the extent of which is dose-dependent. Saxitoxin binding to sodium channels is reversible. The toxin is soluble in water and stable. Dispersal as an aerosol is feasible. Fatalities in adults are reported following ingestion of between 0.5-12.4 mg. Minimum lethal doses in children are estimated to be 25 µg kg⁻¹. (A2.15, A2.16)

Main clinical features

Reported clinical symptoms describe the outcome of ingestion of saxitoxin. Onset of symptoms is typically within 10-60 minutes. Numbness or tingling of the lips and tongue (attributable to local absorption) spreads to the face and neck, followed by a prickling feeling in fingers and toes. With moderate to severe exposure, the parasthesia spreads to arms and legs. Motor activity is reduced, speech becomes incoherent, respiration laboured and subjects die either from paralysis or respiratory arrest. The terminal stages may occur within 2-12 hours.

As to inhalation exposure, for which no cases are reported in the medical literature, animal experiments suggest that the entire syndrome is compressed, and that death may occur within minutes.

Diagnosis and detection

Diagnosis is confirmed by detection of the toxin, using ELISA or mouse bioassay, in samples of, for example, stomach contents, water or food.

Medical management

No specific antidotes exist. Treatment is symptomatic. The toxin is normally cleared rapidly from the body via the urine, so that victims who survive for 12-24 hours usually recover. Diuretics may help. Specific antitoxin therapy has been successful in animals.

Prophylaxis

No vaccine against saxitoxin exposure has been developed for human use.

Stability/neutralization

Saxitoxin maintains its activity in water heated to 120°C.
2.4.2 Ricin (A2.04, A2.05)

Ricin is a highly toxic glycoprotein (a lectin) of approximately 65,000 dalton that occurs in the seed of the castor plant, *Ricinus communis*. Ricin consists of two protein chains, the larger (B chain, 34 Kda) attaching to cell surface receptors and facilitating entry of the smaller (A chain, 32 KDa), which affects cellular ribosomal activity. It inhibits protein synthesis in eukaryotic cells. It is toxic by all routes, including inhalation, but least so by ingestion. Horses are the animals most susceptible to ricin, cattle and pigs less so, with ducks and hens the least susceptible. In mice the systemic LD$_{50}$ is 2.7 µg kg$^{-1}$.

Main clinical features

Following exposure there is a latency of many hours, sometimes days. After inhalation, significant lung pathology is evident with increased cytokine concentrations, marked inflammation and pulmonary oedema. Ingestion results in severe gastro-enteritis, often haemorrhagic. Convulsions, shock and renal failure may develop. Nerve cells, the heart and spleen are all affected by ricin. Ricin dust exposure will cause local irritation of eyes, nose and throat. (A2.15). Sub-lethal lung pathology has been described in immunised mice following inhalation challenge to aerosolised ricin. Survivors of a ricin aerosol challenge may, therefore, experience some injury, particularly to the lungs.

Diagnosis and detection

The primary diagnosis would be by the clinical and epidemiological setting. Specific ELISA testing on serum or immunohistochemical techniques for direct tissue analysis can be used to confirm the diagnosis.

Medical management

Management is supportive and should include maintenance of intravascular volume. No antitoxin is yet available.

Prophylaxis

There is no currently approved prophylaxis for human use, though both active immunization and passive antibody prophylaxis have been under study. Formaldehyde toxoids against ricin have been used successfully to immunize rats: toxoid was administered subcutaneously in 3 doses at 3 weekly intervals and prevented deaths in animals exposed to 5 LCt50s by inhalation challenge. (A2.18)

Stability/neutralization

Ricin is soluble in water, in which state it is less stable than dry product. In the dry state it is normally stable at room temperature but denatures at elevated temperature, the stability decreasing with increasing moisture content. (A2.19)
CITED LITERATURE

Note that these citations have yet to be brought into the format and degree of detail expected by WHO


A2.02 18USC §178(2) as of 26 January 1998.

A2.03 BWC/CONF.II/13 dated 30 September 1986, at part II page 3


A2.05 US Army Medical Research Institute of Infectious Diseases, Handbook: Medical Management of Biological Casualties, second edition, August 1996.


A2.09 UN Office of Disarmament Affairs, compilation of declarations of information by BWC states parties in accordance with the extended confidence-building measures agreed at the Third Review Conference, DDA/4-92/BW3 plus Add.1, Add.2 and Add.3: the Form F filed by the Russian Federation, as translated at the WHO.


A2.15 Hazardous Substances Database 1999


