Overview on the Systematics of Biotoxins as Threat Agents

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15.1 Introduction

Biotoxins are neither distinct biological nor chemical agents in a common understanding but can be considered as 'mid-spectrum agents' [1–3]. As a matter of fact, they deserve special attention as a group of threat agents of biological origin with great potential to harm people [4]. There is a broad spectrum of biotoxins that can be used in biowarfare and in bioterrorist attacks. The spectrum of biotoxins ranges from peptides and proteins to alkaloids and other bioactive small molecules [5, 6].

On the one hand, biotoxins differ from chemical threat agents (CTA) since they are almost never produced synthetically, volatile gases or able to be absorbed through the skin. On the other hand, biotoxins differ from classical biological threat agents (BTA) because they do not carry any genetic information like bacteria or viruses. Nevertheless, some biotoxins are extremely toxic threat agents that can be dispersed as aerosols, liquids or as powders and consequently have the potential to create casualties, alteration or breakdown of social life, or economic loss if used in warfare or a terrorist attack [2, 7–9].

The focus of this chapter will be on biotoxins with mass casualty potential. The differences between CTA, biotoxins, and BTW are explained, and strong emphasis will be placed on the classification of these special group of agents. Biotoxins can be grouped into different 'classes' by mechanism of action or organism of origin [2, 10]. Below, the focus will be strictly on the classification according to the organisms of origin since these agents are very heterogeneous molecules. Additionally, the chapter provides a complete overview of biotoxins that have been considered as threat agents at a certain point by different credible international conventions.

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15.2 Biotoxins as Mid-Spectrum Agents

Paracelsus (1493–1541) expressed the toxicology maxim that "*all things are poison and nothing is without poison, only the dose permits something not to be poisonous*". His principle is based on the simple assumption that all substances can be toxic and "*the dose makes the poison*". The famous Paracelsus phrase also applies to biotoxins. Dose is the key parameter in the hazard identification and risk assessment of biotoxins and the harmful effect is associated with their toxic properties.

As chemicals of biological origin, biotoxins possess characteristics of both groups: chemical and biological agents [4]. Biotoxins are always produced by living organisms and have adverse health effects on humans or other organisms [3, 4]. They represent a subset of poisonous substances in general and can lead to a wide variety of pathologies. The diversity of biotoxins is enormous and includes an extremely heterogeneous group of substances from low-molecular-weight compounds to complex macromolecules [11, 12].

There are a number of reasons why some biotoxins should be considered as threat agents. Biotoxins are naturally occurring substances and their biological effects can cause serious injury or even death. That, in combination with the often existing lack of antidotes for post-exposure prophylaxis and treatment, vaccines for pre-exposure prophylaxis or detection methods makes these molecules critical.

Unlike bacteria or viruses, biotoxins are not able to reproduce themselves or to reproduce with the help of host organisms. Biotoxins do not carry the genetic information necessary for their own amplification and, in view of this fact, these substances resemble chemical agents. CTA, however, possess different characteristics than biotoxins and belong to various classes of compounds with distinct physicochemical, physiological, and chemical properties [13, 14]. Due to the diversity of molecular size and composition of biotoxins and the resulting different physicochemical, physiological and chemical properties they are mostly grouped according to the organisms of origin [2, 10].

Moreover, in contrast to classical CTA, almost all biotoxins are substances that have a low vapor pressure at room temperature. Many CTA—but not all—have a high vapor pressure, resulting in a low boiling point, which causes evaporation from a liquid or solid form to the surrounding air [13]. Since biotoxins are almost never volatile, they cannot be dispersed as gas in contrast to many classical CTA. From this physicochemical perspective, biotoxins are more closely related to classical BTA such as viruses and bacteria.

Beside this fact, the production processes of biotoxins are still completely different compared to those of CTA. Biotoxins are almost exclusively produced by living organisms, whereas CTA are per se synthetically manufactured [14, 15].

Another very distinct feature of biotoxins is that they cannot penetrate the intact human skin without the help of other substances. Dimethyl sulfoxide or other molecules can increase the ability of some biotoxins to penetrate through the skin, but most of them are not skin permeable per se. In contrast to that, some CTA—mustard gas for example—are very lipophilic agents, which can penetrate textiles, biological protective clothing, and even the intact skin.

A further very characteristic feature of many BTA agents, including biotoxins, is an active response of the immune system after contact with those substances. Due to their biological origin, biotoxins stimulate immune reactions. A large group of biotoxins are peptides or proteinogenic molecules that can interfere with the human immune system. The adaptive immune system reacts to most foreign biological substances in a specific way, and the next time the same molecule is encountered, the adaptive immune system can respond faster.

Production, volatility, skin permeability, and immunoreactivity enable the approach of a distinction between biotoxins and CTA. There are also several other indicators and selection criteria available to determine the chemical or biological affiliation of biotoxins (e.g., odor, taste).

The number of biotoxins that can be used as mass casualty biological weapon is very limited. On the one hand, some of the highly toxic biotoxins are not very stable and on the other hand, some of less toxic biotoxins cannot be produced in high quantity or delivered to cover large areas or surfaces [2]. Table 15.1 lists the main criteria, which allow a rough assignment of CTA, biotoxins, and BTA agents.

How difficult it is to distinguish biotoxins from CTA and BTA agents is shown by the following examples. Depending to the authorities involved, the protein ricin is considered as CTA or BTA or both. The organism of origin is the castor oil plant (*Ricinus communis*). Neither the molecular weight, the ability to trigger a clear immune response, nor the natural origin indicates ricin to be a CTA. However, the lack of genetic information for reproduction moves ricin into the direction of CTA.

Likewise, some CTA have characteristics of biotoxins or even BTA. Other CTA, however, are considered unambiguously chemical. An example is sarin, one of the most prominent chemical agents. Sarin is an odorless liquid, which can barely penetrate the human skin. This criterion seems to direct sarin to the BTA or biotoxin side. But as a low-molecular-weight molecule of synthetic origin, which can be produced in large quantities, it clearly fulfills the most important criteria of chemical agents. Therefore, sarin is a CTA and differs from biotoxins and classical BTA.

In summary, several criteria exist to distinguish between BTA, CTA, and biotoxins. However, these individual criteria are not a comprehensive list for the description of threat agents. In general they allow a rough classification of biotoxins in a separate agent group. But, not all criteria must necessarily be fulfilled to place a biotoxin into a particular group. Neither is just one single criterion a prerequisite, nor must several criteria automatically lead to a biotoxins grouping. Nevertheless, in general the criteria allow a classification and an objective comparison of most of the CTA, biotoxins, and BTA agents.

15.3 Committees and Bodies Dealing with Biotoxins

Biotoxins vary according to their organism of origin, molecular structure, size and mode of action. As indicated, not all biotoxins can be considered as mass casualty weapons because not all biotoxins can cause death or disease on a large scale. For

Table 15.1 Different criteria for the discrimination of biotoxins from CTA and classical BTA agents like bacteria and viruses

Criterion	CTA	Biotoxin	BTA
Carrier of genetic information	Never	Never	Always
Type of dissemination	Physical state varies (solid, liquid, gas)	Solid or liquid	Solid or liquid
Effect	Immediately	Mostly short latency period	Mostly long infection period
Immune response	Rare	Mostly immune response	Clear immune response
Infectivity	Not infectious	Not infectious	Often infectious
Molecular size	Low-molecular compounds	Heterogeneous substances (low molecular weight compounds to complex macromolecules)	Highly complex molecular structure
Odor	Characteristic odor	Usually odorless	Usually odorless
Origin	Synthetic	Natural	Natural
Production procedures	Mostly less complex	Mostly complex	Complex
Removal	Decontamination	Decontamination	Disinfection
Routes of entry into the body	Varies; All routes are possible	Via aerosol or oral	Via aerosol or oral
Skin/dermal penetration	Often	Very seldom	Usually none
Taste	Often characteristic taste	Mostly tasteless	Tasteless
Toxicity	High	High	Not toxic
Volatility	Often	None	None

Adopted from Franz [2], Madsen [4], Anderson [7]

this reason, different committees discussed the potential of some biotoxins to be used for biowarfare or bioterrorism.

The Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction, commonly known as the Biological Weapons Convention or Biological and Toxin Weapons Convention (BTWC), discussed biotoxins that do not have prophylactic, protective or other peaceful purposes or that can be used for hostile purposes or in armed conflict [16]. The BTWC was the first multilateral disarmament treaty banning a category of biotoxins [16, 17].

Although biotoxins are considered to be biological, they are still toxic chemicals. Hence, biotoxins are also addressed by the Chemical Weapons Convention (CWC). The CWC aims to eliminate an entire category of weapons of mass destruction by prohibiting the development, production, acquisition, stockpiling, retention, transfer or use of chemical weapons—including toxins weapons—by States Parties [18]. The

agents, which are explicitly specified in the convention for monitoring purposes, cover a wide range of compounds and include chemical warfare agents and biotoxins, including key and more distant precursors. These compounds, or families of compounds, are listed in the three schedules of the convention's Annex [19]. Schedule 1 comprises those agents that have been or can easily be used as chemical weapons and which are of limited, if any, uses for peaceful purposes. This list includes two biotoxins: ricin and saxitoxin [19].

Along with the international conventions on biological and chemical weapons, the US Centers for Disease Control and Prevention (CDC) have prepared a strategic plan for bioterrorism preparedness and response. The plan includes a list of selected agents with putative impact for the public health system. These critical CDC Bioterrorism Agents/Diseases were classified into three Categories: A, B or C. Categorization was based on different criteria like transmission capabilities, severity of morbidity and mortality, and likelihood of use [20]. Many of these agents, in particular biotoxins, are capable to contaminate food or water supplies.

Biotoxins can be found in CDC Categories A and B. Category A agents are the highest priority agents and include *Clostridium botulinum* toxin. This biotoxin is considered to pose a risk to national security as it can easily be disseminated and cause high lethality, with potential for major public health impact. An attack with this toxin might also cause public panic and social disruption and hence requires special action for public health preparedness [20]. Those biotoxins are supposed to be moderately easy to disseminate and cause moderate morbidity and low lethality. Category B agents are the second highest priority agents and include the plant toxin ricin. This biotoxin is considered moderately easy to disseminate; results in moderate morbidity rates and low mortality rates and requires specific enhancements of CDC's diagnostic capacity and enhanced disease surveillance [20].

Another plurilateral like-minded committee addressing questions on BTA and CTA including biotoxins is the Australia Group (AG). All of the participants of the AG are states parties to the BTWC [21]. The AG is an informal forum which, through the harmonization of export controls, seeks to ensure that exports do not contribute to the development of chemical or biological weapons. Coordination of national export control measures assists AG participants to fulfil their obligations under the CWC and the BTWC to the fullest extent possible [21]. One of the group's goals is to agree on agents which are critical for chemical and biological weapons proliferation programs.

Several additional national war weapons lists exist but there is no room to present all of them here (e.g., German *Kriegswaffenliste*, EU CBRN Action Plan). However, all of these conventions and lists (including the ones mentioned above) share a joint understanding and agree on the mass casualty potential of distinct biotoxins. To summarize, only around twenty biotoxins out of millions are considered as mass casualty biological weapons capable of causing death or disease on a large scale. Table 15.2 gives an overview of all of this high risk biotoxins.

Table 15.2 Biotoxins of high risk biological agents lists of the (not adopted) control protocol for the BTWC, the CWC, the AG, and the CDC

Biotoxins	Organism of origin	Class	Listed
Abrin	Rosary pea (*Abrus precatorius*)	Plant toxin	AG, BTWC
Aflatoxin	*Aspergillus flavus* among others	Mycotoxin	AG
Anatoxin	*Cyanobacteria*	Phycotoxin	BTWC
Botulinum toxin	*Clostridium botulinum* among others	Bacterial toxin	AG, BTWC, CDC
Bungarotoxin	Kraits (*Bungarus* snakes)	Venom	BTWC
Cholera toxin	*Vibrio cholera*	Bacterial toxin	AG
Ciguatoxin	*Gambierdiscus toxicus*	Phycotoxin	BTWC
Clostridium perfringens toxins	*Clostridium perfringens*	Bacterial toxin	AG, BTWC
Conotoxin	Cone snails	Venom	AG
Diacetoxyscirpenol	Several fungi	Mycotoxin	AG
Trichothecene toxins	Several fungi	Mycotoxin	AG, BTWC
Microcystine (Cyanoginosin)	Cyanobacteria	Bacterial toxin	AG
Modeccin	Wild granadilla (*Adenia digitata*)	Plant toxin	AG
Ricin	Castor oil plant (*Ricinus communis*)	Plant toxin	AG, BTWC, CDC, CWC
Saxitoxin	*Alexandrium catenella* et al.	Phycotoxin	AG, BTWC, CWC,
Shigatoxin	*Shigella dysenteriae*, *E. coli* among others	Bacterial toxin	AG, BTWC
Staphylococcus aureus toxins	*Staphylococcus aureus* among others	Bacterial toxin	AG, BTWC
Tetanus toxin	*Clostridium tetani*	Bacterial toxin	AG
Tetrodotoxin	Several marine animals	Phycotoxin	AG
Viscumin	Mistletoe (*Viscum album*)	Plant toxin	AG
Volkensin	Kilyambiti plant (*Adenia volkensii*)	Plant toxin	AG

15.4 Classification of Biotoxins

15.4.1 Animal Venoms

Biotoxins and mixtures of them are present in all branches of biological life. A large number of those biomolecule cocktails are found in the animal kingdom and are known as venoms. Animal venoms are heterogeneous blends of toxic substances—mainly of protein and peptide origin—used to hunt for prey or defend against enemies [22]. As a matter of fact, the functional mechanisms of these biological

cocktails are multifaceted and individual compounds of venoms can reinforce each other. Venoms interfere with enzymes, receptors, or ion channels, with impact on the central and peripheral nervous system, the cardiovascular and the neuromuscular system, blood coagulation and homeostasis [23]. In contrast to the harmful effect of venoms, specific compounds of venoms have been increasingly used as pharmacological tools and as prototypes for drug development [24, 25].

The extraction, processing and enrichment of venoms from animals for dissemination and use as threat agent are very challenging. Nevertheless, many of these biotoxins are somewhat accessible and in public perception. Indeed, two zoonotic toxins are listed in the above mentioned international agreements banning biological or chemical weapons: bungarotoxins and conotoxins.

Bungarotoxins are a group of neurotoxic proteins found in the venom of snakes of distinct species, the kraits (*Bungarus* spp.) [26–28]. Four different bungarotoxins are known to interfere with neurological processes: Beta-bungarotoxin acts pre-synaptically, gamma-bungarotoxin antagonizes binding of acetylcholine post-synaptically at peripheral neuromuscular junctions and kappa-bungarotoxin blocks neuronal nicotinic receptors. The most prominent member of the bungarotoxin group is alpha-bungarotoxin. It can lead to headache, unconsciousness, paralysis, respiratory failure, and even death. Alpha-bungarotoxin is a neurotoxin, first described in 1963. It blocks nicotinic acetylcholine receptors and is widely used in medical applications [29–31].

Conotoxins are of special interest for modern pharmaceutical research and are listed for control by the AG. These neurotoxic peptides are derived from cone snail venom and differ between individual snail species. The active components of conotoxins are typically 12–30 amino acid residues in length and act on a wide variety of ligand-gated ion channels leading to various symptoms including paralysis, respiratory failure, and coma [3, 32].

15.4.2 Bacterial Toxins

The biggest group of biotoxins with putative threat potential is the bacterial toxin group. Bacterial toxins can be differentiated into two major classes on the basis of several criteria e.g. their chemical structure, thermostability, and method of release as a pathogen: exotoxins and endotoxins [2, 6, 33].

Endotoxins are structural components of bacteria and part of their cell envelopes. They are bound to the cell wall of gram-negative bacteria and relate specifically to the lipopolysaccharides or lipooligosaccharides located in the outer membrane. Endotoxins may be released from lysed bacteria as a result of effective host defense mechanisms.

Exotoxins are secreted by bacterial cells into the surrounding environment during exponential growth but may also be released during lysis of the cell. The secreted

toxins, soluble proteins or polypeptides, are produced by particular gram-positive or gram-negative bacteria that trigger the disease associated with their respective toxins. All bacterial toxins listed on international agreements banning biological or chemical weapons are protein exotoxins.

Among these very important bacterial toxin group is the so called AB_5 toxin subset [34]. All bacterial toxins of this group contain an enzymatically active A subunit and a homopentameric B subunit which mediates cell entry by oligosaccharide recognition [34–36]. The most prominent AB_5 toxins are **shigatoxins** produced by *Shigella dysenteriae* type 1 and **cholera toxin** produced by *Vibrio cholerae*. Furthermore, **verotoxins** also belong to the group of AB_5 toxins since they are homologous to shigatoxins but produced by enterohaemorrhagic *Escherichia coli* [34, 37–39]. Interestingly, shiga- and verotoxins are structurally closely related to very important biotoxins from plants (e.g., ricin) and are also members of the same ribosome-inactivating protein family (see Sect. 15.4.5).

Further prominent representatives of the exotoxins are the botulinum and tetanus neurotoxins.

Botulinum Neurotoxins (BoNT) are extremely poisonous metabolic products of *Clostridium botulinum* and some other clostridiae and are considered as the most potent natural toxins known [40–48]. *C. botulinum* is a gram-positive, spore-forming rod-shaped bacterium. It grows under the exclusion of oxygen and releases neurotoxins into the surrounding medium.

Six phylogenetic distinct clostridiae are known to produce seven serotypically distinct BoNTs (A-G) [49]. Serotype H was previously discovered but also described as BoNT/FA or BoNT/HA since this serotype seems to be a hybrid of BoNT A und F [50–56]. Types A, B, E, and the rare types F and H are human-pathogenic [57–59].

C. botulinum is widely distributed throughout nature and can occur ubiquitously in soil and mud. Gastrointestinal and cutaneous transmission is possible, respiratory cannot be excluded [60, 61, 62–67]. The main source of human intake of botulinum neurotoxin is contaminated food, mostly meat and sausage products [60]. Depending on the amount of toxin absorbed, symptoms can already appear after a few hours. The toxic effect is caused by irreversible binding to presynaptic nerve endings stopping the release of acetylcholine, thereby disrupting neurotransmission. As a result, neuromuscular transmission is blocked leading to flaccid paralysis.

Tetanus Neurotoxin or tetanospasmin is a poisonous metabolic product of another clostridium: *Clostridium tetani* [68]. The gram-positive spore-forming cells produce the extremely potent neurotoxin under anaerobic conditions. Like *C. botulinum*, *C. tetani* is found throughout nature and can occur ubiquitously in nature. Nowadays, tetanus is a rare disease in the western hemisphere due to excellent vaccination coverage, nevertheless it is still widely distributed in other parts of the world and a major cause of neonatal death in non-vaccinated mothers [69]. The molecular mechanism of action of tetanus toxin results in spastic paralysis [70].

***Clostridium perfringens* Toxins** are other biotoxins with mass casualty potential produced by *C. perfringens*, an ubiquitous bacterium present in the gastrointestinal tract of humans and animals. The gram-positive, anaerobic, endospore forming, and rod-shaped bacteria produce a variety of toxins under anaerobic conditions [71]. These are classified into five 'toxinotypes' (A–E). Each of these toxinotypes is associated with many, often life-threatening illnesses. Especially *C. perfringens* epsilon-toxin, one of the most potent toxins known, is considered as a potential biological weapon and produced by toxinotypes B and D strains [72]. Epsilon-toxin belongs to the heptameric β-pore-forming toxins, which are characterized by the formation of a pore through the plasma membrane of cells, leading to perivascular edema and necrotic lesions causing neurologic signs [73].

***Staphylococcus aureus* Toxins** are biotoxins with mass casualty potential produced by *Staphylococcus aureus* [11]. The gram-positive, round-shaped bacterium can be found everywhere in healthy persons' normal bacterial flora; mostly on the skin, respiratory tract, mucous membranes and in the nose. Nevertheless *S. aureus* can also be very virulent and cause a variety of severe diseases [74, 75]. Some strains are able to produce highly heat-stable protein enterotoxins responsible for symptoms of food poisoning after intake of contaminated food [3]. Staphylococcal food poisoning leads to vomiting, nausea, stomach cramps, and diarrhea within a very short period of time (minutes to hours). The most important staphylococcal enterotoxin which may be used to construct a bioweapon is staphylococcal enterotoxin B (SEB) [3, 4, 76].

15.4.3 Marine Toxins

Marine toxins, also known as phycotoxins, are a very heterogeneous group of biotoxins. They include, for instance, alkaloids, amino acids, and polyketides. They are a class of highly diverse compounds in terms of both structure and biological activity [77]. Phycotoxins can cause various clinically described syndromes, characterized by a wide range of amnesic, diarrheic or azaspiracid symptoms [78]. They cause paralytic shellfish poisonings and ciguatera fish poisoning [78, 79]. Some of these toxins are putative threat agents and almost all members out of this group interfere with neurological processes. They interact with ion channels or receptors, leading to different neurotoxic symptoms and even death. Generally, these types of neurotoxins are marine toxins produced primarily by phytoplankton e.g. flagellates and diatoms, but also by several types of cyanobacteria, invertebrates or other organisms [77].

Most of the phycotoxins that have been considered as threat agents are produced by cyanobacteria (microcystin, anatoxin and saxitoxin). Cyanobacteria—a phylum of bacteria—are ubiquitous photosynthetic microorganisms forming blooms and scums in surface water. Among them, several are known to produce cyanotoxins

giving rise to concern for human health. Cyanobacteria are prokaryotes obtaining energy via photosynthesis. This selling proposition makes cyanobacteria very unique and allows us to separate cyanotoxins from other bacterial toxins.

Microcystines are cyclic peptides produced by a group of cyanobacteria, mostly *Microcystis* spp. Several different microcystins exist and all consisting of a seven-membered peptide ring, which is made up of five non-natural amino acids and two natural amino acids [3]. These natural amino acids distinguishes microcystins from one another, while the other amino acids are more or less constant [3]. Microcystins can cause acute poisonings with a variety of different symptoms and sometimes fatal outcome, but also cancer [80, 81].

Anatoxins are other marine phycotoxins produced by cyanobacteria in the *Anabaena* genus worldwide [82–84]. The most important is anatoxin-a, also known as *Very Fast Death Factor*, which is a secondary amine. Other structurally related alkaloids are homoanatoxin-a, as well as anatoxin-(a)s a unique *N*-hydroxyguanidine methyl phosphate [85–88]. Intoxication by anatoxins results very rapidly in neurotoxic effects, which is specific for this group of phycotoxins.

Saxitoxins are also marine phycotoxins produced by cyanobacteria and dinoflagellates, listed by schedule 1 of the CWC. The saxitoxin-group corresponds to toxic metabolites produced by cyanobacteria and dinoflagellates of the genera *Alexandrium*, *Gymnodinium*, and *Pyrodinium* [89]. Oral uptake of the quite stable saxitoxin and its derivatives can lead very rapidly to paralytic shellfish poisoning including gastrointestinal and neurological signs symptoms [90–92].

Ciguatoxins are a different marine phycotoxin group causing fish poisoning. These toxic polycyclic polyethers are manly produced by the dinoflagellate *Gambierdiscus toxicus* in the Pacific. The dinoflagellates accumulates in fish through the food chain and causes the complex ciguatera clinical picture, including paralysis, heart contraction, and changing the senses of heat and cold. The mechanism of action is the interference of ciguatoxin with voltage-gated sodium channels in synapses of the nervous system [78, 91, 93–95].

Tetrodotoxin is another marine phycotoxin that is considered a potential threat agent [96]. The neurotoxin has been isolated from animals of widely differing species [97]. Tetrodotoxin is well known because of its accumulation in the pufferfish (Fugu), which is a Japan delicacy. The fish must be processed extremely carefully to remove toxic parts containing tetrodotoxin to avoid poisoning. The toxin inhibits the firing of action potentials in neurons by binding to the voltage-gated sodium channels in nerve cell membranes and blocking the passage of sodium ions into the neuron [96]. Symptoms develop very rapidly (within minutes) and include facial and extremity paresthesias and numbness, which may be followed by dizziness and profuse sweating. Death can takes place within a few hours.

15.4.4 Mycotoxins

Mycotoxins are a large group of diverse secondary metabolites produced by a wide variety of filamentous fungi [98]. Up to 400 different molecules are known to be part of the mycotoxin group [99]. Molds of several species may produce the same mycotoxin but sometimes one mold may produce many different mycotoxins [100]. All mycotoxins are low-molecular-weight molecules with the potential to induce toxicological effects in humans and other vertebrates and many mycotoxins display overlapping toxicities to invertebrates, plants, and microorganisms [101]. Mycotoxins are mostly known to cause food poisoning [102].

Trichothecene mycotoxins are produced by several fungi, especially those of the *Fusarium* genus [7, 98]. They have been classified into four groups (Types A, B, C, and D) based on the structure of the molecules [103–105]. Type A-trichothecenes are of special interest in regard to toxicity. They include toxins such as mono- and **diacetoxyscirpenol**, HT-2 toxin, T-2 toxin or neosolaniol [103]. However, some members out of the type B-group also have the potential to harm people in a bioterrorist attack (e.g., deoxynivalenol known as vomitoxin). Trichothecenepoisoning can lead to a variety of clinical signs, including weakness, ataxia, hypotension, coagulopathy, and death [106].

Aflatoxin mycotoxins are a group of chemically similar metabolites produced by certain fungi of the genus *Aspergillus* [98]. Aflatoxins are polycyclic aromatic compounds (difuranocoumarins). Several types are produced in nature and four aflatoxins (B_1, B_2, G_1, and G_2) are naturally found in foods. The predominant site of aflatoxin metabolism is the liver (cytochrome p450 enzymes). There, the biotoxins are metabolized into highly reactive exo-epoxides. Aflatoxin B_1 is most commonly found in food and the most toxic out of the aflatoxin group. Aflatoxins can cause acute poisonings but they are also very potent carcinogens and mutagens casing chronic clinical signs and hepatocellular cancer [107, 108].

15.4.5 Plant Toxins

Extremely toxic biomolecules are biotoxins produced by different plants. Countless plant toxin effects are known since ancient times. Even the father of Greek philosophy, Socrates, died from a plant toxin when he drank a cup of poisonous hemlock. Remarkably, just only a single plant toxin group out of several different has been considered as weapons at a certain point by different committees: the ribosome-inactivating proteins (RIPs) [109].

RIPs are known to be produced by several organisms of all kingdoms: bacteria, fungi, algae, plants, and animals (see Sect. 15.4.2: shiga- and verotoxins). This group of proteins irreversibly modifies ribosomes via their adenine polynucleotide glycosylase activity on different nucleic acid substrates. These modifications are responsible for the arrest of protein synthesis leading to cell death. RIPs have been

classified as type 1, 2, and 3. Type 1 RIPs are single-domain proteins that contain an *N*-glycosidase activity. Type 2 RIPs form a heterodimeric complex consisting of an A-chain and a B-chain linked by disulfide bounds [110, 111]. The A-chain is functionally equivalent to type 1 RIPs (A-chain) but is fused to a C-terminal lectin domain (B-chain). Lectins are glycoside-binding proteins which via lectin-carbohydrate interactions allow the holotoxin to bind to the cell surface. Type 3 RIPs are very rare, only a few of this structurally different RIP types have been classified so far [110, 112, 113].

In general, type 2 RIPs are several times more toxic than type 1 and 3 RIPs, although exceptions are possible (e.g., nontoxic type 2 RIPs) [113, 114]. Only type 2 RIPs, namely abrin, modeccin, ricin, viscumin, and volkensin are agents of concern recognized by committees. Modeccin, viscumin, and volkensin are listed by the Australia Group for export control, but abrin and ricin are considered as dangerous by bodies [115]. Depending on the manner of intoxication, toxicity varies and clinical signs differ.

Ricin is a type 2 RIP produced primarily in the seeds (castor beans) of the castor oil plant (*Ricinus communis*), a member of the spurge family Euphorbiaceae [115]. The plant is native to Africa and cultivated all over the tropical and subtropical world. It is often grown as an ornamental annual in temperate zones and commercially cultivated because of its high amount of oil (castor oil) within the beans which is mainly used in clinical and industrial processes. At the cellular level, ricin hydrolyses the *N*-glycosidic bond of the adenine residue A4324 within the 28S rRNA and leaves the phosphodiester backbone of the RNA intact [116, 117]. Depending on the manner of intoxication, toxicity varies and clinical signs differ. Oral intoxication mostly leads to severe gastrointestinal signs, whereas intoxication by inhalation can cause circulatory instability and severe lung damage.

Abrin is a highly toxic type 2 RIP [115] several times more toxic than ricin. The protein is found in the seeds of the rosary pea (or jequirity pea from *Abrus precatorius*). At the cellular level abrin, causes protein synthesis inhibition at the same site as ricin [118]. Identical RNA *N*-glycosidase activity is present in **modeccin**. This plant type 2 RIP is produced by wild granadilla (*Adenia digitata*) [119]. The fruit and roots are known to be used for suicide. *Adenia* is a genus of flowering plants in the passionflower family, Passifloraceae. The kilyambiti plant (*Adenia volkensii*) is another member of this genus and family that produces a type 2 RIP, **volkensin**, in its roots [120]. Finally, **viscumin** is a toxic type 2 RIP from mistletoe (*Viscum album*) [121].

15.5 Conclusion

Special attention must be paid to ‘mid-spectrum agents’ that pose a serious risk as threat agents or weapons. Besides biotoxins, several other mid-spectrum agents are known. Bioregulators for example are—like biotoxins—on the borderline between

'synthetic' and 'natural' and are neither clear distinct chemical nor biological agents. They are also naturally occurring agents lacking genetic information and are produced by living organisms in order to regulate diverse cellular processes. Like biotoxins bioregulators can have adverse health effects on humans in a short period of time if they are used as biowarfare and bioterrorism agents.

'Mid-spectrum agents' of biological origin have been considered as weapons or instruments of terror. It is impossible to enumerate all molecules of biological origin that have influenced warfare or terroristic efforts or even may be used for such purposes. However it remains to be emphasized that in the case of biotoxins; only around 20 have been discussed in the public by different credible international conventions or bodies as founding substances for weapon capable of causing death or disease on a large scale. Thus, at least these biotoxins ought to be discussed further in regard to challenges and requirements with respect to public health preparedness. The biotoxins discussed in this chapter may serve as the basis for the development of appropriate methods of management and countermeasures, including decontamination and Personal Protective Equipment strategies.

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