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Review

Cyanobacterial toxins – occurrence, biosynthesis and impact on human affairs

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Mass developments of cyanobacteria ("blue-green algae") in lakes and brackish waters have repeatedly led to serious concerns due to their frequent association with toxins. Among these are the widespread hepatotoxins microcystin (MC) and nodularin (NOD). Here, we give an overview about the ecostrategies of the diverse toxin-producing species and about the genes and enzymes that are involved in the biosynthesis of the cyclic peptides. We further summarize current knowledge about toxicological mechanisms of MC and NOD, including protein phosphatase inhibition, oxidative stress and their tumor-promoting capabilities. One biotransformation pathway for MC is described. Mechanisms of cyanobacterial neurotoxins (anatoxin-a, homanatoxin-a, and anatoxin-a(s)) are briefly explained. We highlight selected cases of human fatalities related to the toxins. A special focus is given to evident cases of contamination of food supplements with cyanobacterial toxins, and to the necessary precautions.

Keywords: Cyanobacterial blooms / Food supplements / Microcystin / Nodularin / Toxicity

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1 Introduction

Certain cyanobacteria are able to flourish in aquatic environments where they can produce blooms and scums [1]. Whereas this mass development of cyanobacteria is a source of nuisance both visually and due to released odor factors, concerns that are more serious are related to the toxins produced by certain species of cyanobacteria. The ecological function of cyanobacterial toxins remains under investigation, and toxicity to vertebrates may have occurred as side effect during the evolution [2]. Their toxic mechanisms to vertebrates are nevertheless used to separate them into hepatotoxins (microcystin, MC, and nodularin, NOD), neurotoxins (anatoxin-a, -as, homanatoxin), cytotoxins (cylindrospermopsin), dermatotoxins (lyngbyatoxin), and irritant toxins (lipopolysaccharides) [3]. For the risk assessment of cyanobacterial toxins, a basic understanding of the properties of the producing cyanobacteria, and of their toxicological potential is inevitable. Here, we provide an overview about the occurrence, biosynthesis and toxicology of the most widespread toxin class in cyanobacteria, the cyclic peptides microcystin (MC) and nodularin (NOD) (Fig. 1).

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Abbreviations: **antx-a(s)**, anatoxin-a (s); **antx-a**, anatoxin-a; **MC**, microcystin; **NOD**, nodularin; **NRPS**, nonribosomal peptide synthetases; **PKS-I**, type I polyketide synthases; **PP**, proteine serine/threonine phosphatases; **PSP**, paralytic shellfish poisoning; **ROS**, reactive oxygen species

2 Ecostrategies of toxin-producing cyanobacteria

Harmful algal blooms have been reported not only from freshwater but also from marine environments and have been implicated in a series of human and animal poisonings. Despite of striking similarities between the algal mass developments in the different ecosystems they significantly differ with respect to the organism causing the bloom and to the nature of the toxins produced. Bloom formation in marine environments is often caused by eukaryotic microalgae, in particular by dinoflagellates [4]. Freshwater lakes and brackish water environments such as the Baltic Sea, however, are often dominated by cyanobacteria (Fig. 2). Botanists have originally considered cyanobacteria as "blue-

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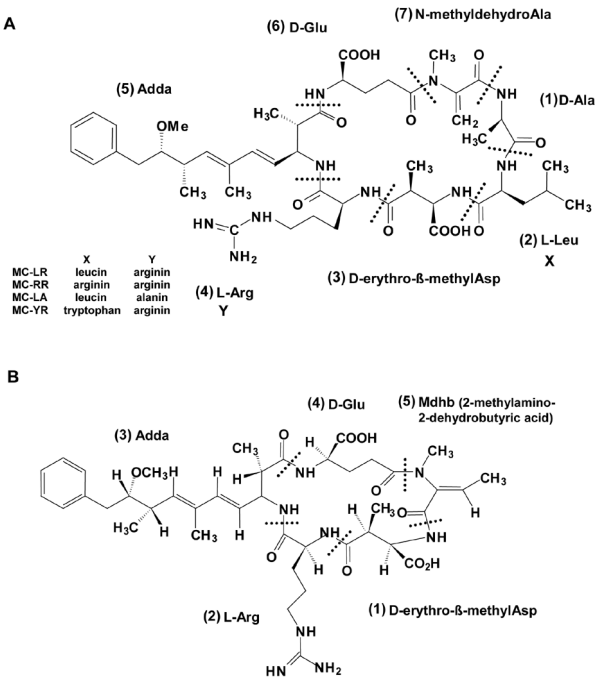

Figure 1. (A) Structure of the hepatotoxin microcystin-LR. The amino acids at position 2 (X) and 4 (Y) are variable. Examples for the main microcystin congeners are indicated. (B) Structure of the hepatotoxic nodularin.

green algae", a term that is still used in the public media. This apparent confusion of names reflects the fascinating combination of properties exhibited by these organisms. Whereas the photosynthetic apparatus of cyanobacteria is similar to algae, genetically these organisms must be considered as true bacteria. The lysis of a cyanobacterial bloom leads to the release of high amounts of blue-pigmented proteins, the so-called phycobiliproteins. These blue pigments enable cyanobacteria to use a wider light spectrum than terrestric land plants and have provoked their early description as blue-green algae. Cyanobacteria are important primary producers and play a crucial role in their ecosystems [5]. Thus, their harmful features must be considered also in context with their beneficial properties.

The diversity of cyanobacteria is reflected by variations in their cell morphology, in metabolic strategies, their motility, differences in their cellular differentiation, *etc*. Ecologists typically observe a seasonal succession of phytoplankton in lakes. Whereas at the beginning of a summer a great variety of cyanobacteria and eukaryotic algae co-exist in lakes, this diversity drastically drops towards the end of a summer as the result of the mass development of one cyanobacterial genus. Frequently, mass developments are observed for *Microcystis, Planktothrix, Anabaena* and *Nodularia.* These bloom-forming cyanobacteria are predominant producers of MC and NOD, respectively.

A common feature of MC and NOD-producing cyanobacteria is the presence of gas vesicles. They are small and hollow air filled structures of cylindrical shape that provide buoyancy [6]. Gas vesicle enable the bacteria, after periods of water mixing, to float up from the deeper water layer back into the euphotic zone, where light for photosynthesis is provided, or to reach deeper nutrient-rich layers by sinking. Therefore, these organisms have means to overcome spatial separation of nutrition and light. The ability to regulate their buoyancy is discussed as a major advantage over other phytoplankton species and may partly explain the enormous success of the toxin-producing species in the field. Despite these similarities individual toxin producing genera have different ecostrategies and inhabit different ecosystems.

Microcystis is a unicellular colony-forming cyanobacterium that preferentially occurs in water bodies deeper than

Figure 2. Scum of *Microcystis* at the shore of Lake Wannsee in the Berlin area (2005).

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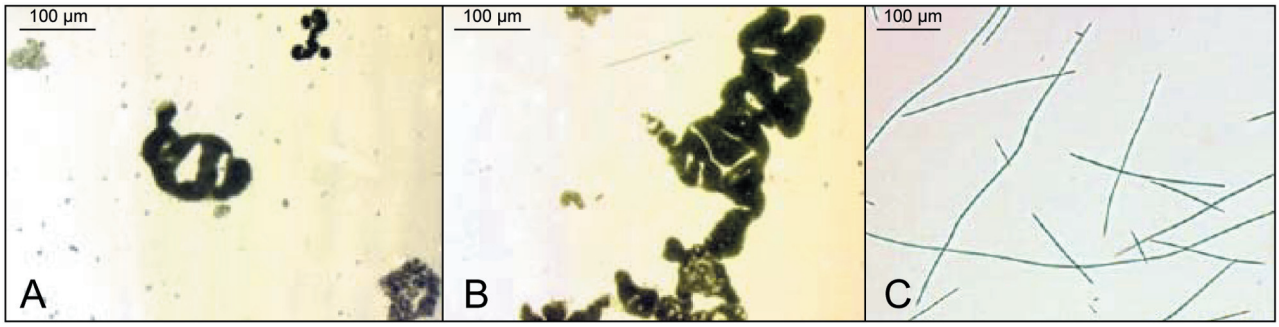

Figure 3. (A and B) *Microcystis* morphotypes co-existing in a field sample of Lake Wannsee. (C) Filaments of a toxic *Planktothrix agardhii* strain from a laboratory culture.

3 m that provide optimal conditions for the vertical migration. *Microcystis* colonies can be assigned to different morphotypes that differ in their shape and sheath characteristics, but also in the size of their individual cells [7]. *Microcystis* blooms normally consist of a mixture of different morphotypes (Fig. 2A and B). Eventually, all colonies may become buoyant and accumulate on the water surface where they can be blown together by the wind, forming stable scums (Fig. 3). A similar behavior is observed for the filamentous cyanobacteria *Anabaena* and *Nodularia* [7]. Both genera are able to differentiate specialized cells for nitrogen fixation, so-called heterocysts, and are in particular well adapted in lakes with periodic nitrogen limitation. *Anabaena* blooms were most frequently reported from freshwater lakes in Nordic countries [8]. *Nodularia* has been implicated in toxic bloom formation in brackish and estuarine environments, particularly in the Baltic Sea [8]. In contrast to the scum-forming ecostrategists, *Planktothrix agardhii* filaments are homogenously dispersed in the water column. These cyanobacteria are very sensitive to high light conditions and mostly inhabit turbid shallow lakes [1] (Fig. 2C). The closely related species *Planktothrix rubescens* possesses additional red pigments, the phycoerythrins and develops stable summer populations in the metalimnion zone of thermally stratified lakes. At the end of a growing season, the filaments can become buoyant and may form red surface scums [9]. The MC and NOD producing cyanobacterial blooms are frequently contaminated or mixed with cyanobacteria producing additional toxins, in particular with those producing the neurotoxins anatoxin-a and saxitoxin. The latter is better known as paralytic shellfish poisoning (PSP)-toxin and predominantly produced by dinoflagellates in marine ecosystems, however also by cyanobacterial species, namely *Aphanizomenon flos aquae* and *A. gracile* [10, 11]. Anatoxin-a is produced by the cyanobacteria *Anabaena flos-aquae* and *A. circinalis,* but also by species of the genera *Aphanizomenon*, *Cylindrospermum*, *Planktothrix* [12–14] and in *Microcystis aeruginosa* [15].

3 Biosynthesis of cyclic peptide toxins

MC biosynthesis has been first elucidated in two *Microcystis* strains [16–18]. The *mcy* gene cluster in *Microcystis* encodes six multienzymes that can be assigned to the family of nonribosomal peptide synthetases (NRPS) and type I polyketide synthases (PKS-I), respectively (Fig. 4). Nonribosomal peptides (NRP) and polyketides are a structurally diverse group of secondary metabolites that have been identified in a number of prokaryotes and lower eukaryotes [19, 20]. The wide varieties of structures have an equally wide range of bioactivities, including antibiotic (*e.g.* erythromycin A or penicillin), antifungal (*e.g.* amphotericin), anticancer (*e.g.* epothilone) and immunosuppressive (*e.g.* cyclosporine or rapamycin) properties. The biosynthesis of the highly diverse structures is achieved on NRPS/PKS-I templates that have a modular architecture. One module comprises a set of domains that are responsible for the activation, modification and elongation of a single amino acid or carbon unit (Fig. 4). NRPS and PKS enzymes accept a wide range of different substrates. Moreover, some of the enzyme units show flexibility for different substrates. This property of the enzymes can at least partly explain the diversity of MC that were detected even in the same strain. In addition to the broad specificity of the enzymes, however, recombination events have led to altered substrate specificities of domains involved in MC biosynthesis, thus contributing to the evolution of diverse toxin forms [21]. As a result, more than 80 isoforms of MC were reported that could also significantly differ in their toxicology. The discovery of MC biosynthesis genes in *Microcystis* has paved the way not only for the identification and for elucidation of the MC pathways in the filamentous cyanobacterial genera *Planktothrix* and *Anabaena* but has also led to the discovery of the enzymes involved in NOD biosynthesis in *Nodularia* [22–24]. As expected, the similarity in structure between the heptapeptide MC and the pentapeptide NOD (Fig. 1) is reflected by a similar mechanism of biosynthesis (Fig. 4).

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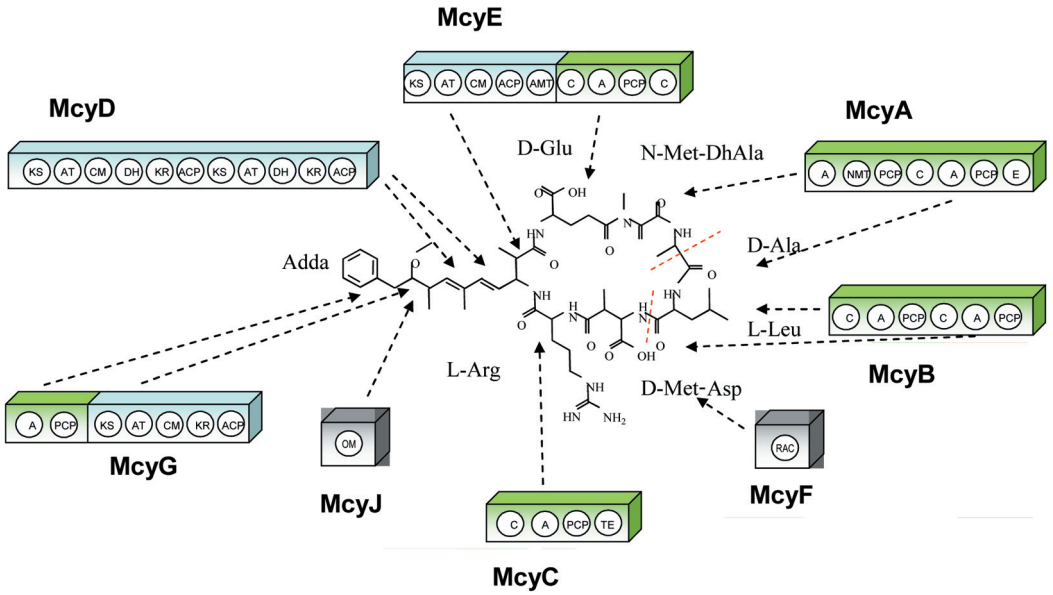

Figure 4. Model of the domain structures of multienzymes involved in microcystin (MC) and nodularin (NOD) formation. KS: β-ketoacyl synthase, AT: acyltransferase, ACP: acyl carrier protein, KR: ketoacyl reductase, DH: dehydratase, CM: C-methyltransferase, OM: O-methyltransferase, NM: N-methyltransferase, AMT: aminotransferase, RC, racemase. Polyketide synthase (PKS) domains are indicated in blue, nonribosomal peptide synthetases (NRPS) domains are indicated in green, and tailoring proteins are indicated in grey boxes. Red lines are bordering the two amino acid moieties lacking in the NOD structure.

The phylogenetic comparison of the toxin biosynthesis genes in the individual genera has allowed reconstructing their evolutionary history. Distances in the sequences of the related genes in the individual genera indicated a very long and independent evolution of the MC and NOD genes, rather than an ongoing horizontal gene transfer between the genera. These analyses have further indicated that a common ancestor of the genes has already existed very early in evolution, long time before the eukaryotic predators appeared on earth [2]. For all MC and NOD producing genera, strains have been described that contain the toxins genes and strains that lack the genes and accordingly the ability to produce the toxins. In the field, these toxic and nontoxic strains normally co-exist. However, toxic and nontoxic strains cannot be differentiated by morphological criteria or by classic molecular techniques. Thus, molecular detection techniques based on the toxin biosynthesis genes were established both for the identification and for the quantification of toxic field strains. *Mcy* genes of unknown origin can be assigned to the producing organism. For field monitoring, colony- or filament-based techniques have been developed that overcome the quantitative biases of selective strain isolation (for a review see [25]). Finally, quantitative PCR techniques have been developed that allow a quantification of the toxin biosynthesis genes [26, 27]. These molecular techniques can assist direct chemical measurements that are needed for a risk assessment of MC and NOD in the environment. Furthermore, molecular techniques will allow studying population dynamics of toxigenic cyanobacteria in the future and will thus help us understanding the success of these organisms in lakes and ponds.

4 Toxicological mechanisms

4.1 Hepatotoxicity and protein phosphatase inhibition

All structural congeners of MC and NOD act as hepatotoxins, because after ingestion by vertebrates, they accumulate from the small intestine into the liver due to the active uptake by an unspecific organic anion transporter, known as bile acid carrier transport system. Damage of liver cells include cytoskeletal disorganization, lipid peroxidation, loss of membrane integrity, DNA fragmentation and strand breaks, cell blebbing, apoptosis, cellular disruption, necrosis, and intrahepatic bleeding, which may lead to death of the organism by hemorrhagic shock [28–35]. Despite that in vertebrates the liver is the main suffering organ, the molecular mechanisms of MC or NOD is the same in all cells, regardless of the organism, with the only exception of the cyanobacteria themselves.

MC and NOD both are cyclic peptides consisting of seven (MC) or five (NOD) amino acids. Structural variations occur by changing of two (MC) or one (NOD) amino acid, and several changes in small side groups. A common char-

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acteristic of both hepatotoxins is the rare amino acid Adda that is the responsible structure for the specific inhibition of protein serine/threonine phosphatases (PP1 and PP2A, [29, 36]). Protein phosphatases are blocked if Adda interacts with the catalytic site of the enzyme [36]. The covalent binding of MC by cysteine (Cys-273 of PP-1 or Cys-266 of PP2A) provides additional stability of the complex, but is not required for inhibition of the protein phosphatases [37–39]. Molecular changes of the Adda moiety, therefore, immediately affect the toxicity of MC [40–42]. In contrast to MC, NOD does not bind covalently to PP1 or PP2A [43].

In a broad selection of organisms, including plants, zooplankton, bivalves and vertebrates, protein phosphatases inhibition by MC-LR varies only in the IC_{50} range of 0.1–0.25 nM [29, 44, 45]. In contrast, cyanobacterial protein phosphatases show a much lower sensitivity, despite most of the amino acids required for its catalytic function are conserved [46]. Through PP inhibition, both toxins increase the phosphorylation of cellular proteins. Hyperphosphorylation of the intermediate filaments of the cell, cytoceratin 8 and 18, is then the main cause for changes in whole-cell morphology as a result of cytoskeletal rearrangements [47, 48]. In vertebrate livers, contact with neighboring cells is reduced and sinusoidal capillaries loose stability, which rapidly leads to intrahepatic hemorrhage and often results in serious liver malfunction or death [3].

4.2 Tumor-promoting capacity

A severe aspect of MC and NOD toxicity is their tumor promoting capacity. Stimulation of preneoplastic cells by MC mainly occurs in the liver, the accumulating organ [40], but has also been detected in the colon, where the non-resorbed MC remains and metabolites are transported via bile fluid [49]. Whereas the previous studies used diethylnitrosamin as tumor initiator, tumor-promoting activity of MC-LR was also demonstrated in rats after initiation by the natural mycotoxin aflatoxin B_1 [50]. If cyanobacterial extracts are used instead of purified MC, the situation is more realistic and even worse: its genotoxicity has been proven using different test systems, Ames test for mutagenicity using *Salmonella typhimurium*, or by the detection of ouabain-resistant mutation using cultured human RSa cells, comet assay for DNA aberration, and micronucleus test for damage to DNA or mitotic apparatus [51, 52]. These findings evidenced the health risk of deprived people, which due to their poverty lack the access to proper food and clean drinking water.

NOD proved to be an even stronger promoter, compared to MC, and additionally caused a slight increase of initiated cells itself [53].

The mechanism behind tumor promotion by MC might again be attributed to PP inhibition, as the tumor suppressor gene products, retinoblastoma and p53, are both inactivated by phosphorylation. Hyperphosphorylation of the two proteins and consequently proliferation of previously initiated cells was observed after application of ocadaic acid, a marine toxin produced by dinoflagellates of the species *Dinophysis* and *Prorocentrum*, acting via the same PP inhibition mechanism [54].

4.3 Oxidative stress

In addition to protein phosphatase inhibition, the cells suffer from oxidative stress caused by MC. Oxidative stress generally occurs via the formation of reactive oxygen species (ROS), which include superoxide and hydroxyl radicals, and radicals of cellular organic compounds, causing damages such as peroxidation of lipids, proteins, and DNA. The formation of ROS by MC results in lipid peroxidation, membrane damages, and lactate dehydrogenase leakage from the cells or loss of mitochondrial membrane potential [34, 52, 55–59]. Mitogen-activated protein kinases (MAPK), in particular those involved in the p53 pathway, seem to mediate the response to cyanobacteria and their toxins in the dinoflagellate *Peridinium gatuense* and influence the kinetics and intensity of internal ROS formation that functions as signal for apoptosis in older cells [60].

On the gene level, increasing ROS leads to an oxidative degradation of DNA [32, 34, 35]. DNA fragmentation started with oxidation of purines that seem not to undergo repair, but even provoked further DNA strand breaks [61]. Oxidation of DNA furthermore resulted in the formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine [62]. Thus it was concluded, that long-term exposure to MC-LR might cause genotoxicity and carcinogenicity mediated by ROS. In human lymphocytes, MC-LR treatment induced DNA damage in a time-dependent manner, without changing the frequency of chromosome aberrations. It was concluded that the observed DNA damage is more likely related to the early stages of apoptosis and thus to cytotoxicity but not to genotoxicity [63]. An inhibiting effect of MC on the repair of radiation-induced damage [63] is also supported by mentioned above recent findings from Zegura *et al*. [61].

5 Biotransformation

Protection against oxidative damage of the cell can be provided by the membrane antioxidant vitamin E or selenium pre-treatment, the latter supporting the selenium-dependent glutathione peroxidase, an antioxidative enzyme [64–66]. Accordingly, oxidative damage of the DNA was completely prevented by ROS scavengers and partly diminished by

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hydroxyl radical scavengers [61]. Furthermore, antioxidative enzymes, including superoxide dismutase, catalase, peroxidase, and glutathione-peroxidase prevented oxidative damages via reduction of the oxidized metabolites [58, 59, 67–70].

Biotransformation of both MC and NOD in aquatic organisms (fish, mussels, daphnids, and macrophytes) starts by conjugation to the intracellular tripeptide glutathione by glutathione-S-transferase [71]. By conjugation, the water solubility is enhanced compared to the parent compound and so conjugation to glutathione aids excretion of the toxins. The conjugation product has also been identified in rodents and birds [72–74]. As conjugation to glutathione appears at the methyl group of N-methyl-dehydroalanine (Mdha, Fig. 1), opposite to the Adda moiety, the conjugate is still capable of inhibiting PP. The MC-LR-glutathione S-conjugate is further metabolized by gamma-glutamyltransferase and dipeptidases to remove the glutamyl and glycyl moieties, and yield a MC-LR cysteine S-conjugate [75]. As a result, inhibition of PP increases again with each metabolic step, but stays weaker compared to the non-conjugated toxin [76]. MC metabolites and non-metabolized MC can be transported from the liver either to the body via the blood stream, or back to the small intestine via the bile fluid. From the bile fluid of rainbow trout, MC or its metabolites have been isolated, which were still capable of PP inhibition [77].

Via the blood stream, MC and MC conjugates are distributed in the body to other organs such as muscles, and even the brain [78–82]. Filtration from the blood may explain why kidneys are affected as well, suffering from apoptosis and from morphological degeneration of Bowman's capsules and kidney tubuli [83, 84].

Glutathione is required for biotransformation of the toxin, and as co-substrate for the antioxidative enzyme glutathione peroxidase. Its protective capacity has been demonstrated by Hermansky *et al*. (1991), whereas its depletion or oxidation limits detoxifying capacity of the organisms [85–88].

The adenosine triphosphate synthase β-subunit has been identified as a further binding protein for MC-LR affecting the capability of cellular energy restoring [89].

The mechanisms described above emerge during chronic exposure, despite low doses.

6 Human fatalities

Despite animals being more often involved, reports of human intoxication with cyanobacteria originate from all countries experiencing blooms of toxic cyanobacteria (for detailed examples see, *e.g*. [90, 91]). Chances of intoxication increase with bloom density and towards the end of a season and with the lysis of the cells.

Microcystin intoxication mainly involves the following exposure routes and sources of the toxin: (i) by consumption of toxin-contaminated drinking water, especially if surface water was used as drinking water resource; (ii) during recreational use of water bodies suffering from blue green algae mass developments, by skin contact, unintentional drinking and inhalation; (iii) by deliberate consumption of blue green algae containing food and food supplements, with the possibility of contamination by toxic species; (iv) by hemodialysis if the water for the medium was improperly purified.

Symptoms caused by MC can include nausea/vomiting, weakness, skin irritation, and illnesses ranging from gastroenteritis [92–100], and pneumonia [32] to hepatoenteritis [101]. The long list of examples for gastro-enteritis starts in 1931 along the Ohio River, infested by a cyanobacterial bloom [92]. Seasonal cases involve either summer activities [97–99] or use of water bodies as drinking water supply during the lysis of the bloom [94]. Insufficient or wrong treatment, *e.g.* (induced lysis of the cells) during drinking water purification, belongs to the main threat and cause of intoxications and deaths [95, 96, 99, 101, 102]. The most fatal intoxication known to date occurred via hemodialysis treatment using inadequately purified water for medium preparation, in Caruaru, Brazil, where in summer 1996 a mass development of cyanobacteria occurred in the drinking water reservoir, amongst them *Microcystis*, *Anabaena* and *Cylindrospermopsis* species. Hemodialysis patients suffered from hepatic and gastrointestinal damage, and 76 of the 131 treated died within 20 months. High concentrations of the cyanobacterial toxins MC and cylindrospermopsin were found in the liver [103].

Consequences of chronic exposure to low concentrations of MC are unequivocally discussed, because accumulation, biotransformation, and elimination have to be considered, but the main concern arises from their tumor-promoting capacity. Epidemiological studies found a correlation between primary liver cancer (PLC) and the use of untreated surface water as drinking water resource in combination with hepatocarcinogens such as the mycotoxin aflatoxin B_1 from mould or infection by hepatitis B virus: A systematic screening of drinking water resources in China suggested that the combination of the cyanobacterial toxin, detected in drinking water originating from pond and ditch waters, and the hepatocarcinogen aflatoxin B_1 from mould cereals, may contribute to the high incidence of primary liver cancer in this region [104–106]. A similar study in Florida found elevated risk of primary hepatocellular carcinoma in residents near surface-water treatment plants com-

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pared to areas where ground water is available for drinking water purposes [107].

Furthermore, intoxication can arise via bioaccumulation in food-organisms, mainly fish or mussels from cyanobacterial-contaminated waters [78, 108] but also from plants irrigated with cyanobacteria-containing waters [109].

No intoxication risk for humans seems to exist via bioaccumulation in cattle fed with cyanobacteria, neither in milk nor in the beef [110–112].

7 Further cyanotoxins

7.1 Anatoxins

Anatoxins are the structurally closely related alkaloids anatoxin-a and homoanatoxin-a, and the phosphate ester of a cyclic N-hydroxyguanidine structure, anatoxin-a(s), Fig. 5 [12, 113].

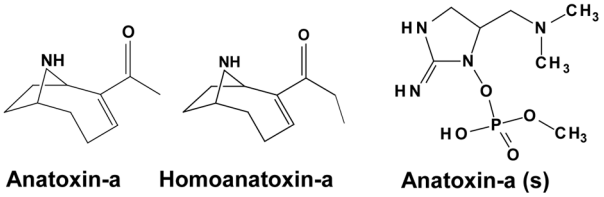


Figure 5. Structures of the neurotoxins anatoxin-a, homoanatoxin-a, and anatoxin-a (s).

Anatoxin-a, a nicotinic agonist, mimics the neurotransmitter, acetylcholine, by irreversible binding to the nicotinic acetylcholine receptor of the sodium channel of the postsynaptic neurotransmitter plate. Anatoxin-a(s) blocks the acetylcholinesterase, so that after a signal transmission, acetylcholine is not removed from the nicotinic receptor by cleavage into its two component parts, acetic acid and choline [114]. By both mechanisms, the postsynaptic sodium channel of the following neuron is permanently opened, and inflowing sodium ions generate action potentials till energetic exhaustion of the nerve cell. Consequently, the muscles are over-stimulated, tetanus occurs, and might be followed by fatigue. Oxygen supply is disrupted, if respiratory muscles are affected by anatoxin-a, first affecting the brain functions and causing convulsions, followed by suffocation.

Intoxications of vertebrates by anatoxin-a or anatoxin-a(s) often include dogs after ingestion of cyanobacterial contaminated waters [14, 115], or herbi, respectively, planktivorus birds [116, 117].

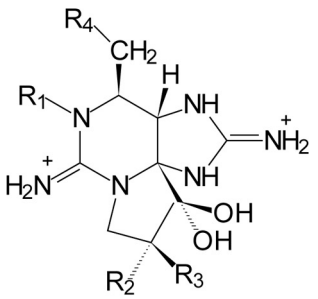
7.2 PSP toxins

PSP toxins (Fig. 6) are a family of more than 20 derivatives of a molecule consisting of a tetrahydropurine group and two guanidinium moieties. PSP toxins are assembled into carbamoyl or carbamate toxins (saxitoxin, neosaxitoxin, gonyautoxins 1–4), their N-sulfocarbamoyl derivatives (gonyautoxins 5, and 6, and C1–4) and decarbamoyl toxins, plus small modifications of those resulting from sulfatation at positions R2 and R3 (Fig. 6) [118].

By means of binding to the voltage-gated Na^+ channels in nerve cells PSP toxins block neuronal transmission leading to muscle paralysis and death by respiratory arrest in mammals [119]. The guanidinium group at positions C-7, -8 and -9 and the hydroxyl group at C-12 are essential for binding to the Na^+ channels, with toxicity increasing with the net charge of the molecule [120]. Clams and crabs can bioaccumulate PSP toxins in high concentrations, and appear to be insensitive to them, but posing risk to their predators and humans [121, 122]. Receptors for PSP toxins discovered in the circulatory fluids of aquatic animals such as amphibian, fish, reptiles, and arthropods, seem to provide protection from the toxin [123].

8 Toxic cyanobacteria and food supplements

Blue green algae products attract consumers' attention for their putative improvement of general health or well being, manifested as improved memory and elevated attention,



R_1	R_2	R_3	R_4 : $OCNH_2$	R_4 : $OCNOSO_3^-$	R_4 : OH	R_4 : H
H	H	H	STX	GTX 5	dcSTX	doSTX
OH	H	H	neoSTX	GTX 6	dcneoSTX	-
OH	OSO_3^-	H	GTX 1	C 3	dcGTX 1	-
H	OSO_3^-	H	GTX 2	C 1	dcGTX 2	doGTX 2
H	H	OSO_3^-	GTX 3	C 2	dcGTX 3	doGTX 3
OH	H	OSO_3^-	GTX 4	C 4	dcGTX 4	-

STX: saxitoxin, GTX: gonyautoxin, dc: decarbamoyl, do:deoxycarbamoyl

Figure 6. Structures of paralytic shellfish poisoning toxins.

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Table 1. Acute toxicity of cyanobacterial toxins intraperitoneally applied to mouse, their mode of toxic action and producing cyanobacteria

Cyanobacterial toxin	LD_{50} (μg/kg mouse i.p.)	Toxic mechanism	Reference
Microcystin-LR	50	PP inhibition	[133]
Microcystin-YR	70	PP inhibition	[133]
Microcystin-RR	600	PP inhibition	[3]
Anatoxin-a	200–250	Mimics the neurotransmitter acetylcholine	[3]
Anatoxin-a(s)	20	Blocks acetylcholinesterase	[3]
Saxitoxin	10	Blocks voltage-gated Na^+ channels	[3]
Nodularin	50	PP inhibition	[134]

increased energy and immune status, relief from exhaustion, nervousness, depression and premenstrual syndrome. Blue green algae food supplements (tablets, capsules) are even used against Attention Deficit Disorder in children. These supplements comprise a huge variety of vital nutrients, such as all amino acids, including the essential ones, numerous trace minerals, omega-3 and -6 essential fatty acids, β-carotene, vitamins (B12, K), and chlorophyll. Blue green algae products mainly originate from *Spirulina* sp. or *Aphanizomenon flos aquae* cultures. Both culturing in farms and harvesting from natural lakes exist.

Potential risks of exposure to cyanobacterial toxins in blue green algae products principally arise from contamination of these cultures with toxin-producing strains or from confusion with toxin-producing species. Furthermore evidence increases, that also *Spirulina* itself might be capable of synthesizing the cyanobacterial neurotoxin anatoxin-a that has been detected in *Spirulina* products [124].

Contamination by toxin-producing strains may have led to the detection of MC in blue green algae products in 85 of 87 samples tested. Of these samples, 72% contained more than 1 μg of MC/g, exceeding the levels considered to be safe by the World Health Organization (WHO) [125]. MC-LR was also the main congener found in more than 100 blue green algae products analyzed in a second study [126]. Routine measurements of both the harvest and the product are necessary to minimize or exclude intoxication risks, with sensitive methods for measurements being available [127]. Especially in harvests from natural lakes, where variation in composition of the species occurs, this can lead to different contamination of each shipment. The degree of risk for each person also depends on doses and duration of exposure to the toxin. Children are at particularly risk because of their lower body weight, and keeping in mind, that these food supplements are recommended for treatment of Attention Deficit Disorder.

The WHO published a provisional guideline value of 1.0 μg MC-LR/l in drinking water, based on acute toxicity testing using swine and mouse [128–130]. However, this provisional guideline is calculated without regarding the tumor-promoting capacities of MC, and should therefore include an additional uncertainty factor of three, leading to a guideline value of 0.3 μg/L [131]. Still, Dietrich and Hoeger [132] highlighted deficiencies of the data used for the risk assessment underlying the guideline and their application: all emphasis lies on only 1 of the nearly 80 congeners of MC, the MC-LR. New knowledge about toxic mechanisms, such as potential neuro- and renal toxicity is excluded. In addition, exposure scenarios including blue green algae food supplements especially in children would be necessary.

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9 References

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