ACTA BIOLOGICA CRACOVIENSIA Series Botanica 45/2: 9–20, 2003

NATURAL TOXINS FROM CYANOBACTERIA

JOANNA MANKIEWICZ[2,3*], MAŁGORZATA TARCZYŃSKA[2], ZOFIA WALTER[1], AND MACIEJ ZALEWSKI[3]

[1]*Department of Molecular Genetics and* [2]*Department of Applied Ecology*
University of Łódź, ul. Banacha 12/16, 90-237 Łódź, Poland
[3]*International Centre for Ecology, PAS, ul. M. Konopnickiej 1, 05-092 Łomianki, Poland*

Received April 5, 2002; revision accepted November 22, 2002

Morphologically, physiologically and metabolically, cyanobacteria (blue-green algae) are one of the most diverse groups of prokaryotes. Cyanobacteria bloom abundantly in surface waters as a result of eutrophication, and they produce different types of toxins, so they not only hinder recreational use of bodies of water but also cause health problems in humans and animals. Cyanobacterial toxins (cyanotoxins) can be classified in five groups: hepatotoxins, neurotoxins, cytotoxins, dermatotoxins and irritant toxins (lipopolysaccharides).

Key words: Cyanobacteria, blue-green algae, cyanotoxins, effect on health.

INTRODUCTION

Cyanobacteria are gram-negative photosynthetic prokaryotes. They can be found in a wide range of habitats from ice fields to hot springs and deserts. Morphologically, physiologically and metabolically, this group is one of the most diverse groups of prokaryotes (Codd, 1994). The rapid evolution of cyanobacteria in different water and land environments is related to their capacity for both aerobic and anaerobic photosynthesis. They contain chlorophyll-*a*, carotene, xanthophyll, blue *c*-phycocyanin and red *c*-phycoerythrin. The last two pigments can only be found in cyanobacteria (Benson, 1969; Ressom et al., 1994; Duy et al., 2000). Their photosynthetic organ and mechanism of photosynthesis are similar to algae but, unlike eucaryotic microalgae, cyanobacteria do not possess membrane-bound subcellular organelles like chloroplasts. The photosynthetic pigments of cyanobacteria are located in thylakoids lying free in the cytoplasm near the cell periphery.

One of the fundamental metabolic processes of cyanobacteria is dinitrogen fixation. Using the enzyme nitrogenase, they convert N_2 directly into ammonium in aerobic conditions. Nitrogen-fixing cyanobacteria are widespread among filamentous, heterocyst-forming genera such as *Anabaena, Nostoc, Aphanizomenon* (Ressom et al., 1994; Adams, 2000). Some non-heterocyst forming species such as *Trichodesmium* can also assimilate nitrogen (Carpenter et al., 1992).

Cyanobacteria bloom intensively in eutrophic surface waters (lakes, reservoirs and slow rivers). This natural process of enrichment of biological production in aquatic ecosystems is caused by increases in the level of nutrients, usually phosphorus and nitrogen compounds. Some lakes are naturally eutrophic, but in many other water bodies the excess nutrient input is of anthropogenic origin, resulting from municipal wastewater discharge or runoff from agricultural land.

Cyanobacteria have a number of special properties that determine their importance, relative suc-

* e-mail: mankiew@biol.uni.lodz.pl.

PL ISSN 0001-5296

© Polish Academy of Sciences, Cracow 2003

Fig. 1. *Microcystis* bloom in Sulejow Reservoir. **Fig. 2**. *Microcystis aeruginosa* scum (0.5 m thick) appearing in Sulejow Reservoir in September 1999.

cess and predominance during the growth season in phytoplankton communities. However, the behavior of different cyanobacterial taxa is not homogeneous because their ecophysiological properties differ (Mur et al., 1999). Cyanobacterial blooms are usually observed during springtime (*Planktotrix rubescens*, *Limnotrix redekei*) or during late summertime (*Microcystis aeruginosa*, *Aphanizomenon flos-aquae*, *Planktotrix agardhii*). The following factors are responsible for the predominance of bloom-forming cyanobacteria during the summer period: water temperature above 25˚C, low light intensity in water, low N:P ratio, and stability of the water column.

Many species of cyanobacteria possess gas vesicles, which enable regulation of the buoyancy of cells and colonies, and optimize their vertical position in the water column; this in turn enables them to find a suitable niche for survival and growth. The buoyancy of some cyanobacteria is responsible for intensive formation of scum at the water surface (Figs. 1, 2). The slow growth rate of cyanobacteria in comparison to eucaryotic microalgae is compensated by a higher affinity for phosphorus and nitrogen, substantial phosphorus storage capacity, and low losses to grazing by zooplankton as a result of the formation of large colonies (Reynolds, 1987; Tarczyńska et al., 1997).

Cyanobacterial blooms are unattractive and they hinder recreational use of water bodies, but more important are the health problems their toxins cause in humans and animals (Carmichael, 2001). Contact with or ingestion of water containing cyanobacterial cells or toxins can cause skin irritations, allergic responses, blistering of mucosa, hay fever symptoms, diarrhoea, acute gastroenteritis, and liver and kidney damage (Ressom et al., 1994; Falconer, 1994b; Bell and Codd, 1996; Pilotto et al., 1997; Codd, 2000).

The cyanobacterial toxins (cyanotoxins) can be classified in five groups: hepatotoxins, neurotoxins, cytotoxins, dermatotoxins and irritant toxins (lipopolysaccharides) (Tab. 1). In the aquatic environment these toxins usually are contained mainly within the cyanobacterial cells, and are released in substantial amounts during cell lysis (Sivonen and Jones, 1999).

HEPATOTOXINS

Cyclic peptides represented by two groups of cyanotoxins are included among the hepatotoxins: microcystins and nodularins. These are produced mainly by the genera *Microcystis*, *Planktothrix*, *Anabaena*, *Nodularia*, *Nostoc* and *Umezakia* (Carmichael, 1994, 1997; Harada, 1994; Codd, 1995; Sivonen, 1996; Falconer, 1999; Singh et al., 1999; Chorus et al., 2000; Codd, 2000).

Microcystins differ from nodularins in amino acid content. Both these groups of hepatotoxins contain a unique hydrophobic amino acid, ADDA (2S,3S,8S,9S-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-deca-4,6-dienoic acid) (Eriksson et al., 1987). The ADDA chain is indispensable to the biological activity of these molecules; changes in the ADDA chain reduce the toxicity of hepatotoxins (Carmichael, 1992; Stotts et al., 1993; Dow and Swoboda, 2000; Duy et al., 2000).

Microcystins consist of seven amino acids. The name of these hepatotoxins is derived from the first genus found to produce it, *Microcystis aeruginosa* (Fig. 3) (Carmichael, 1992; Falconer, 1999; Singh et al., 1999). At least 75 microcystin variants are

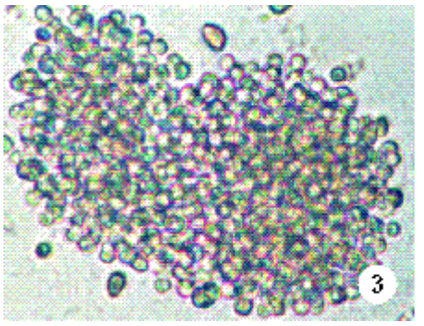

Fig. 3. Colony of *Microcystis aeruginosa*. × 400.

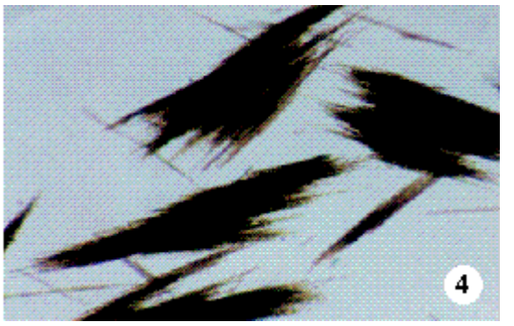

Fig. 4. Aggregates of *Aphonizomenon flos-aquae*. × 400.

known (Sivonen, 1996; Chorus and Bertram, 1999; Codd, 2000; Carmichael, 2001). Microcystins differ from each other by the L amino acid at two non-conserved positions in the molecule (Botes et al., 1982; Ressom et al., 1994; Duy et al., 2000). This was the basis for naming the different microcystin variants such as microcystin-LR (Fig. 5), which contains the most common L-amino acids, leucine (L) and arginine (R); microcystin-YR, which contains leucine (L) and tyrosine (Y); and microcystin-RR, which contains two arginines (RR) (Carmichael et al., 1988; Duy et al., 2000). Different types of microcystins are formed by substitutions at amino acid sites by methylation or demethylation of the molecule, by variations in the structure of the ADDA chain, or by modification of D-glutamic acid (Bell and Codd, 1996; Duy et al., 2000).

Nodularins (Fig. 6) represent the second group of hepatotoxins; they consist of five amino acids, and apart from the ADDA chain contain D-glutamate (D-Glu), L-arginine (Arg), D-β-methylaspartic acid (MeAsp) and D-*N*-methyl-dehydrobutyrin (Mdhb)

TABLE 1. Cyanobacterial toxins: their function and mechanism of action (according to Chorus and Bartram, 1998; Falconer, 1999; Chorus et al., 2000; modified)

Toxin	Primary target organ in mammals	Taxon	Mechanism of toxicity
HEPATOTOXINS			
microcystins	Liver	*Microcystis, Oscillatoria / Planktothrix, Nostoc, Anabaena*	Inhibition of protein phosphatase activity, hemorrhaging of the liver
nodularins	Liver	*Nodularia*	
CYTOTOXINS			
cylindrospermopsins	Liver, kidney, spleen, intestine, heart, thymus	*Cylindrospermopsis, Umezakia*	Inhibition of protein synthesis
NEUROTOXINS			
anatoxin-a	Nerve synapse	*Anabaena, Oscillatoria, Aphanizomenon*	Blocking of post-synaptic depolarization
anatoxin-a(s)	Nerve synapse	*Anabaena*	Blocking of acetylcholinesterase
saxitoxins	Nerve axons	*Aphanizomenon, Anabaena*	Blocking of sodium channels
neosaxitoxins	Nerve axons	*Aphanizomenon, Anabaena*	
DERMATOTOXINS			
lungbyatoxins-a	Skin	*Lungbya*	Protein kinase C activators, inflammatory activity
debromoaplysiatoxins	Skin	*Lungbya*	
aplysiatoxins	Skin	*Lungbya, Schizothrix, Oscillatoria*	
IRRITANT TOXINS			
lipopolysaccharides	Any exposed tissue	All	Potential irritant and allergen

(Rinehart et al., 1988; Duy et al., 2000). At least seven variants of nodularin from the filamentous cyanobacterium *Nodularia spumigina* have been isolated (Lahti 1997; Chorus and Bartram, 1999; Falconer, 1999; Codd, 2000).

Microcystins and nodularins show similar biological activity in spite of their different chemical structures. They are hepatotoxic to animals and people. Hepatotoxins cause disruption of liver structure by hypovolemic shock and excessive blood pooling in the liver (Carmichael, 1992; Premazzi and Volterra, 1993; Ressom et al., 1994; Singh et al., 1999).

Like the other cyanotoxins, hepatotoxins may enter the body through oral consumption, inhalation or skin absorption (Falconer, 2001). Toxicity by oral uptake is generally the least, because > 90% of microcystin is excreted (Falconer 1988; Chorus et al., 2000). Hepatotoxins cannot be destroyed by digestion, and they are transported through the gastrointestinal tract to the liver (Falconer, 1999). The hepatotropism of microcystins is due to selective uptake by liver cells through the bile acid transport system specific to this organ (Eriksson, 1990; Eriksson et al., 1992). They inhibit specific protein phosphatase enzymes type 1 or 2 (PP1 or PP2A) within the liver cells, which are key components controlling the cell structure and function (MacKintosh et al., 1990; Runnegar et al., 1993; Bell and Codd, 1994; Toivola et al., 1994; Singh et al., 1999; Codd, 2000). Microcystins bind covalently with PP1 or PP2A. Goldberg et al. (1995) demonstrated that microcystin-LR (MC-LR) is bound to cysteine-237 on PP1 via the *N*-methyl dehydroalanine of microcystin. Specific binding of microcystin to protein phosphatases induces increased overall phosphorylation of several cytosolic and cytoskeletal proteins (Eriksson et al., 1990; Toivola et al., 1994; Toivola and Eriksson, 1999). Toivola et al. (1997) established that two of the major MC-LR-induced hyperphosphorylated proteins are liver intermediate filament (IF) proteins, keratin 8 (K8) and keratin 18 (K18). Keratins interact with desmoplakin in a phosphorylation-dependent way, and as desmoplakin reactivity disappears the keratin filaments at cell borders start to withdraw towards more central parts of the cells (Toivola et al., 1997; Toivola and Eriksson, 1999). Additionally, MC-LR also induces time-dependent disassembly of microtubules (MTs), and this effect is mediated by hyperphosphorylation

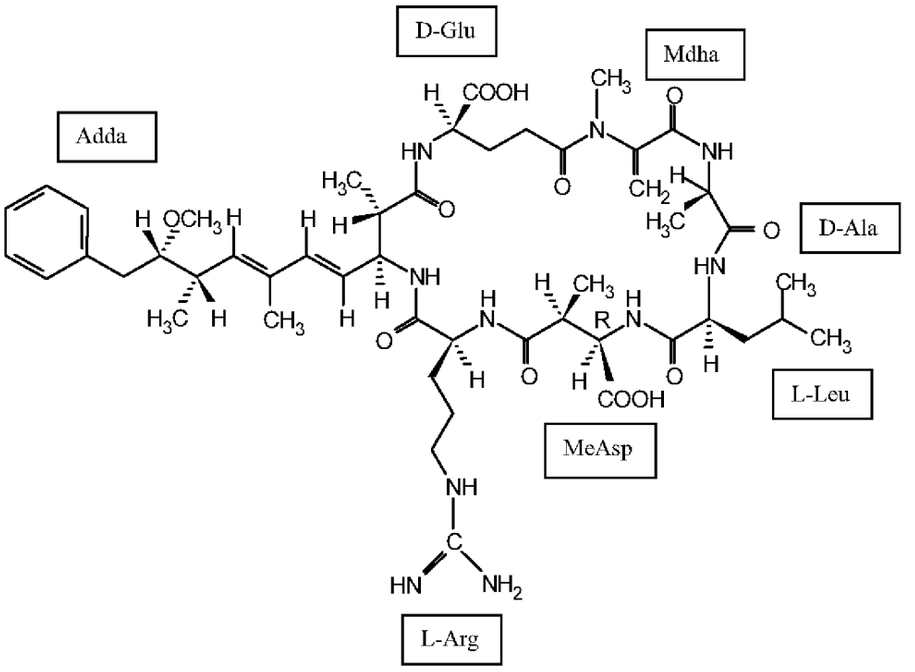

Fig. 5. Chemical structure of microcystin-LR. D-Ala – D-alanine; L-Leu – L-leucine; MeAsp – D-erythro-β-methyloaspartic acid; L-Arg – L-arginine; Adda – 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-deca-4,6-dienoic acid; D-Glu – D-glutamate; Mdha – N-methylodehydroalanine.

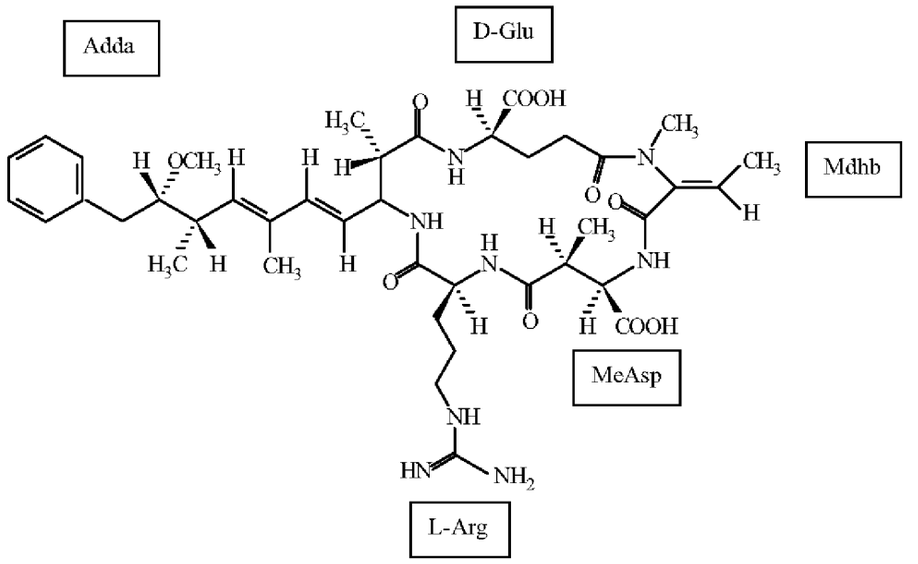

Fig. 6. Chemical structure of nodularin. MeAsp – D-erytro-β-methyloaspartic acid; L-Arg – L-arginine; Adda – 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-deca-4,6-dienoic acid; D-Glu – D-glutamate; Mdhb – N-methyldehydrobutyrin.

of some of the microtubule-associated proteins (MAPs) (Toivola et al., 1994; Toivola and Eriksson, 1999). Hyperphosphorylation of cytosolic and cytoskeletal proteins damages cell structures and causes cytoskeletal disintegration. One of the first observations of intracellular effects is the dramatic formation of plasma membrane blebs and reorganization of F-actin (Eriksson et al., 1989b; Mills et al., 1998).

Inactivation of protein phosphatase by hepatotoxins disturbs the normal balance of cell processes, resulting in cell proliferation and cancer production, or apoptosis and cell death (Boe et al., 1991; Fujiki and Suganuma, 1993; Toivola et al ., 1994; Fladmark et al., 1998; Mankiewicz et al., 2001). These two different effects on the cell depend on the dose and duration. When cells are exposed to a single dose of 1.0 nM MC-LR, or multiple doses of MC-LR at pM concentrations, cytokinesis is stimulated and the rate of apoptosis is reduced (Humpage and Falconer, 1999). This could be connected with the tumor-promoting activity of microcystin. A higher (10 nM) concentration of MC-LR inhibits cytokinesis and induces programmed cell death (Humpage and Falconer, 1999). Additionally, nodularin can activate several proto-oncogenes of the *fos* and *jun* family, which are considered to play a role in tumor promotion (Ohta et al., 1994).

Cyanobacterial poisons can affect not only hepatocytes but also other types of cells. Microcystins and nodularins can inhibit the activity of PP1 and PP2A in any eucaryotic cells they can get into, but usually need the bile acid carrier to cross cell membranes. Apoptotic changes have been observed after microinjection of microcystins or nodularins into human embryo kidney HEK 293, Swiss 3T3 mouse embryo fibroblast, breast carcinoma cell line MCF-7 and rat promyelocytic IPC-81 leukemia cells (Fladmark et al., 1999). Morphological changes such as membrane budding, cell shrinkage and organelle redistribution have been seen in human fibroblasts, human endothelial cells, human epithelial cells, human lymphocytes and rat promyelocytes (McDermott et al., 1998; Mankiewicz et al., 2000, 2001). Microcystins also have a clastogenic effect in human lymphocytes, connected with a dose-related increase of chromosomal breakage (Repavich et al., 1990; Premazzi and Volterra, 1993).

CYTOTOXINS

One cytotoxin from cyanobacteria which is highly toxic through both oral consumption and injection is cylindrospermopsin (Falconer, 1999). It is a tricyclic alkaloid, possessing "a tricyclic guanidine moiety

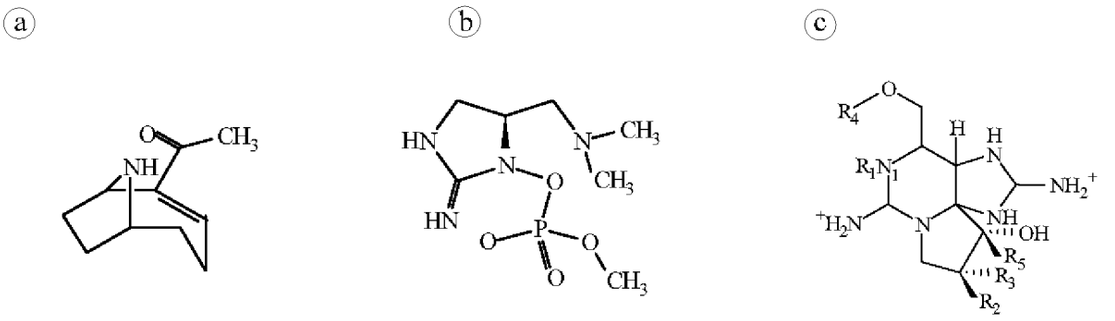

Fig. 7. Chemical structure of (**a**) Anatoxin-a, (**b**) Anatoxin-a(s), (**c**) Saxitoxin.

combined with hydroxymethyluracil"; cylindrospermopsin is biosynthesized mainly by *Cylindrospermopsis raciborskii* and *Umezakia natans* (Ohtani and Moore, 1992; Chorus and Bartram, 1999; Falconer, 1999; Duy et al., 2000). This cytotoxin blocks protein synthesis generally (Hawkins et al., 1985, 1997; Chorus et al., 2000). The first clinical symptoms of poisoning are kidney and liver failure; it also causes damage to the spleen, intestine, heart and thymus (Chorus et al., 2000; Codd, 2000). Cylindrospermopsin is especially dangerous because clinical symptoms may become manifest only several days after exposure, so the toxic effects can be difficult to correlate.

NEUROTOXINS

Neurotoxins are one of the most toxic compounds produced by cyanobacteria. Because the species responsible for neurotoxin production are not common, these toxins have a narrower impact than hepatotoxins. Moreover, not so many populations of the potential producers actually produce the toxins. Neurotoxins are produced by species and strains of the genera *Anabaena* (Carmichael et al., 1990), *Aphanizomenon* (Fig. 4) (Mahmood and Carmichael, 1986), *Nostoc* (Davidson, 1959) and *Oscillatoria* (Sivonen et al., 1989; Skulberg et al., 1992). Neurotoxins interfere with the functioning of the neuromuscular system. Paralysis of peripheral skeletal muscles and respiratory muscles causes death within minutes (Ressom et al., 1994; Singh et al., 1999). At present, four groups of neurotoxins have been well described: anatoxin-a, anatoxin-a(s), saxitoxin (Figs. 7 a,b,c), and neosaxitoxin (Ressom et al., 1994; Chorus and Bartram, 1999).

Anatoxin-a from *Anabaene flos-aquae* was the first neurotoxin characterized. Anatoxin-a is an alkaloid with a low molecular weight of 165 daltons (Carmichael, 1992). It is a secondary amine, 2-acetyl-9azabicyclo(4-2-1)non-2-ene (Huber, 1972). The structure and action of anatoxin-a is similar to acetylcholine (Spivak et al., 1980; Ressom et al., 1994; Dow and Swoboda, 2000). Anatoxin-a is a potent post-synaptic depolarizing neuromuscular blocking agent. In general, this neurotoxin brings about its effect through acetylcholine and the related enzyme acetylcholinesterase. In a normal situation, acetylcholine is released by neurons innervating muscle cells, and binds to the acetylcholine receptor. After acetylcholine binds to the receptor, the ion channel is opened and muscle cells are activated. The acetylcholine is degraded by acetylcholinesterase, the ion channel is closed, and the muscles rest. Anatoxin-a disturbs this process. After it binds to the receptor, it cannot be degraded by acetylcholinesterase, and the muscle cells are overstimulated. Paralysis of the respiratory muscles ensues, followed by suffocation and death (Ressom et al., 1994; Falconer, 1999; Singh et al., 1999).

Anatoxin-a(s) is structurally unrelated to anatoxin-a, but the effects of the two neurotoxins are similar. Anatoxin-a(s) is a unique N-hydroxyguanidine methyl phosphatase ester with a molecular weight of 252 daltons (Matsunaga et al., 1989; Ressom et al., 1994). This neurotoxin inhibits the enzyme acetylcholinesterase. The biomolecular reaction occurs initially with the formation of an enzyme-anatoxin-a(s) complex, which results in phosphorylation of the enzyme. In this condition the enzyme cannot degrade acetylcholine, which remains continuously available to stimulate and overstimulate the muscle cells (Carmichael, 1994; Singh et al., 1999). A symptom of anatoxin-a(s) poisoning is excessive salivation (Carmichael and Falconer, 1993).

Neosaxitoxins and saxitoxins (potent paralytic shellfish poisons, PSP) are unique tricyclic mole-

cules with hydropurine rings (Ressom et al., 1994). They are produced by the genera *Anabaena* and *Aphanizomenon*. These neurotoxins inhibit nerve conduction by blocking sodium channels only; they do not affect the flow of potassium or the resting potential of the membrane or membrane resistance (Adelman, et al. 1982; Gorham and Carmichael, 1988). These sodium channel-blocking agents inhibit transmission of nerve impulses and the acetylcholine cannot be released. The effects of neosaxitoxins or saxitoxins can lead to death by respiratory arrest (Carmichael and Falconer, 1993; Duy et al., 2000).

DERMATOTOXINS

Dermatotoxins, which include aplysiatoxins, debromoaplysiatoxins and lyngbyatoxins, are produced mainly by tropical and subtropical marine benthic cyanobacteria such as *Oscillatoria, Lyngbya* and *Schizothrix* (Chorus and Bartram, 1999). Toxic action connected with dermatitis is characteristic of aplysiatoxins and debromoaplysiatoxins, which are potent tumor promoters and protein kinase C activators (Mynderse et al., 1977; Fujiki et al., 1990). Lyngbyatoxin-a causes dermatitis and oral or gastrointestinal inflammation (Cardellina et al., 1979).

IRRITANT TOXINS – LIPOPOLYSACCHARIDES (LPS)

Generally, lipopolysaccharides are an integral component of the cell wall of all gram-negative bacteria, including cyanobacteria. They can elicit irritant and allergenic responses in human and animal tissues that come into contact with these compounds (Chorus and Bartram, 1999). LPS from cyanobacteria can also cause gastroenteritis and inflammation, but they are less toxic than LPS from pathogenic gram-negative bacteria such as *Salmonella* (Bell and Codd, 1996). The toxicity mechanism of LPS endotoxins produced by cyanobacteria is still largely unknown (Duy et al., 2000).

HEALTH EFFECTS OF CYANOTOXINS

Many strains and species of cyanobacteria produce toxic compounds that can pose major problems in recreational and drinking water supplies, causing livestock death and human intoxication. Exposure to toxins from cyanobacteria is expected to influence both morbidity and mortality (Falconer, 2001). For example, long-term oral consumption of *Microcystis* Kutzing toxins by mice has been demonstrated to cause chronic active liver damage and, in the same experiment, increased mortality from respiratory disease (Falconer et al., 1988). Moreover, epidemiological evidence of increased rates of primary liver cancer in a specific population in China has been linked to consumption of cyanobacteria-contaminated, untreated surface water (Yu, 1995).

Over a century ago, George Francis (1878) published the first documented case of lethal intoxication of livestock by drinking water from an Australian lake heavily infested with cyanobacterial blooms. Since then, cyanotoxins have been shown to be dangerous to various groups of wild and domestic animals such as sheep, cattle, horses, pigs, dogs, cats, monkeys, birds, fish, rodents, amphibians, waterfowl, bats, zebras, rhinoceros and invertebrates (Codd et al., 1989; Gunn et al., 1992; Carmichael and Falconer, 1993; Duy et al., 2000; Chorus et al., 2000). The effects of cyanobacterial toxins on animals include hepatotoxicosis and neurotoxicosis. The hepatotoxic symptoms in animals include weakness, reluctance to move, anorexia, pallor of the extremities and mucous membranes, and mental derangement (Carmichael and Falconer, 1993; Duy et al., 2000). The neurotoxins can cause progression of muscle fasciculations, decreased movement, abdominal breathing, cyanosis, convulsions and death (Carmichael and Falconer, 1993; Duy et al., 2000).

Cyanobacterial toxins also present hazards to human health, but the evidence linking them to human illnesses is still open to criticism. The reservations have to do with the difficulty of identifying and quantifying cyanobacterial toxins in health incidents. Epidemiological study is required to explore whether a relationship exists between human illnesses and cyanobacterial contamination of water.

The first recorded case of human illness was an outbreak of gastroenteritis associated with cyanobacterial toxins occurring in the Ohio and Potomac Rivers in 1931 (Tisdale, 1931). That year a massive *Microcystis* bloom caused illness in 5,000 to 8,000 people who drank water from these rivers. The methods used to treat the drinking water, such as precipitation, filtration and chlorination, were insufficient to remove the cyanotoxins. Unfortunately, the species and toxins responsible for this episode were not identified. Subsequent years have yielded much evidence of human intoxication after consumption of drinking water containing cyanotoxins.

As an example, in 1979 in Australia, 139 children aged 2–16 years and 10 adults experienced a hepatitis-like syndrome with malaise, anorexia, vomiting, tender hepatomegaly, headaches and abdominal pain after drinking water from an open reservoir where *Cylindrospermopsis raciborskii* as a source of cylindrospermopsin production was identified (Byth, 1980; Hawkins et al., 1985).

Yu's research from 1995 on the epidemiology of human hepatocellular carcinoma in China implicated cyanobacterial toxins as part of a complex of agents that increase that disease (Falconer, 1999). Studies showed that people who drank pond and ditch water had a death rate of 121 per 100,000, compared with zero for those who drank uncontaminated water. Epidemiological studies in China also indicated that the risk of primary liver cancer was about 8 times higher in people who drank pond and ditch water than in those who drank uncontaminated water (Singh et al., 1999).

The recreational use of lakes and rivers is the next most common route of exposure to cyanobacterial toxins. As an example, in 1959 in Canada, 12 people became ill with headaches, nausea, muscular pains and acute gastroenteritis symptoms (Dillenberg and Dehnel, 1959). The illness was noted in people who before intoxication had been swimming in a lake contaminated by toxins from *Anabaena*. In England in 1989, 10 of 20 soldiers became ill after swimming and canoe-training in water with a heavy bloom of *Microcystis* (Turner et al., 1990); 2 cases of pneumonia were noted among the soldiers.

Healthy people are less susceptible to cyanotoxicosis than people with hepatitis, alcoholism or kidney damage. Kidney dialysis patients are especially susceptible to toxic damage from cyanobacteria. In 1996 in Caruaru, Brazil, 131 dialysis patients were exposed to microcystins via water used for dialysis; 56 of them died, and the others had typical symptoms of harm from microcystins such as nausea, vomiting and painful, excessive enlargement of the liver (Jochimsen et al., 1998). The episode of deaths of hemodialysis patients at the Caruaru dialysis clinic emphasized the importance of hemodialysis water as an exposure route for microcystins (Jochimsen et al., 1998; Codd, 2000).

An additional aspect influencing the toxicity of cyanobacterial blooms is the age of the victim. Children are more vulnerable for several reasons: they drink more water per unit of body weight, are less likely to have a choice of the source of drinking water, and are more susceptible to damage that takes a considerable time to develop, such as environmentally induced carcinomas (Falconer, 1999).

Bioaccumulation of peptide toxins in food chains may be another important factor. There is only limited information about the accumulation of microcystins and nodularins in aquatic organisms. They can accumulate in, for example, freshwater mussels (*Mytilus edulis*), freshwater clams and fish such as flounder (*Platichthys flesus*) or salmon, and can transfer through the food chain (Eriksson et al., 1989a; Rabergh et al., 1991; Prepas et al., 1997; Duy et al., 2000; Sipia et al., 2001). The high filtration capacity of mussels feeding on blue-green algae irrespective of any toxicity implies that mussels can transfer large doses of toxins to birds, fish and humans (Falconer, 1994a; Vasconcelos, 1995). Whether the levels of microcystin accumulation are sufficient to pose a risk to humans is still uncertain, and will depend on levels of consumption and the severity of toxic blooms in areas where fish or shellfish are caught or collected (Chorus and Bartram, 1999).

Dense cyanobacterial blooms may harm aquatic plant germination and establishment (Casanova et al., 1999). The presence of microcystins in water used for irrigation can have a considerable impact on the growth and development of crop plants (Pflugmacher et al., 1998; McElhiney et al., 2001). Microcystins inhibit growth and development in potato shoots and mustard seedlings under laboratory conditions. The findings suggest that exposure to these hepatotoxins via irrigation water contaminated with toxic cyanobacteria pose a threat to the quality and yield of crop plants in the environment (McElhiney et al., 2001). On the other hand, *Aphanizomenon flos-aque*, which can produce cyanotoxins, is used as a food supplement. This cyanobacterium is harvested from a natural lake, Upper Klamath, and quality control issues regarding the presence of cyanotoxins is always needed (Carmichael et al., 2000). Unfortunately, we have not enough knowledge of the influence of long-term consumption of drinking water contaminated by cyanotoxins. There may be a real threat to human health connected with the consumption of water containing barely detectable doses of toxins, as well as with the use of contaminated water for food production. In view of the toxic and genotoxic effect of cyanotoxins, in 1997 the World Health Organization established 1 μg/l microcystin-LR or microcystin-LR equivalents as a guideline for acceptable levels of cyanotoxins in drinking water.

To counter the potential impact of blooms and cyanotoxins on drinking water quality and public health, new, more efficient water treatment technol-

ogy has been implemented. The efficiency of elimination of cyanotoxins dissolved in water by conventional treatment methods such as coagulation, flocculation or rapid sand filtration is very low, not exceeding 11–18% (Duy et al., 2000; Tarczynska et al., 2001). The use of powdered (PAC) and granulated activated charcoal (GAC) adsorption can achieve 90–100% removal of cyanotoxins following conventional water treatment (Keijola et al., 1988). The use of activated charcoal has expanded in recent years, but it is still not a widely used method because of its high cost. The most consistently efficient process for destruction of both intra- and extracellular cyanotoxins appears to be ozonation, which destroys these toxic compounds rapidly (Rositano et al., 1996; Hart et al., 1997). Chlorination could also be very effective, but this process depends strictly on the pH and dissolved organic carbon (DOC) concentration in the water (Nicholson et al., 1994; Hart et al., 1997). Care must be taken with chlorination procedures to deal with the release of chlorine into the air and the formation of excess levels of trihalomethanes (Hrudey et al., 1999).

ACKNOWLEDGEMENTS

This work was supported in part by grants from the State Committee for Scientific Research (6PO4F 083 21 and 7T09D 01321), the Mayor of the City of Łódź (2000/38, G-51) and PROJECT EC-EVK2-2001-00182.

REFERENCES

ADAMS DG. 2000. Heterocyst formation in cyanobacteria. *Current Opinion in Microbiology* 3: 618–624.

ADELMAN WJ, FOHLMEISTER JF, SASNER JJ, and IKAWA M. 1982. Sodium channels blocked by aphantoxin obtained from the blue-green algae *Aphanizomenon flos-aquae. Toxicon* 20: 513–516.

BELL SG, and CODD GA. 1994. Cyanobacterial toxins and human health. *Reviews in Medical Microbiology* 5: 256-264.

BELL SG, and COOD GA. 1996. Detection, analysis and risk assessment of cyanobacterial toxins. In: Hester RE, Harrison RM [eds.], *Agricultural chemicals and the environment*, 5, 109–122. Royal Society of Chemistry, Cambridge, UK.

BENSON HJ. 1969. A laboratory manual in general microbiology. *Microbiological application*. 2nd ed. Brown, Company Dubuque, IA.

BOE R, GJERSTEN BT, VINTERMYR OK, HOUGE G, LANOTTE M, and DOSKELAND SO. 1991. The protein phosphatase inhibitor okadaic acid induces morphological changes typical of apoptosis in mammalian cells. *Experimental Cell Research* 195: 237–246.

BOTES DP, KRUGER H, and VILJOEN CC. 1982. Isolation and characterization of four toxins from the blue-green algae, *Microcystis aeruginosa. Toxicon* 20: 945–954.

BYTH S. 1980. Palm Island mystery disease. *Medical Journal of Australia* 2: 40–42.

CARDELLINA JH, MARNER FJ, and MOORE RE. 1979. Seaweed dermatitis structure of lyngbyatoxin A. *Science* 204: 193–195.

CARMICHAEL WW. 1992. Cyanobacteria secondary metabolites-the cyanotoxins. *Journal of Applied Bacteriology* 72: 460–466.

CARMICHAEL WW. 1994. The toxins of cyanobacteria. *Scientific American* 270: 64–70.

CARMICHAEL WW. 1997. The cyanotoxins. In: Callow JA [ed.], *Advances in botanical research*, 211–256. Academic Press, London, UK.

CARMICHAEL WW. 2001. Health effect of toxin-producing Cyanobacteria: "The CyanoHABs". *Human and Ecological Risk Assessment* 7: 1393–1407.

CARMICHAEL WW, and FALCONER IR. 1993. Disease related to freshwater algal blooms. In: Callow JA [ed.], *Advances in botanical research*, 187–210. Academic Press, London, UK.

CARMICHAEL WW, BEASLEY V, and BUNNER DL. 1988. Naming of cyclic heptapeptide toxins of cyanobacteria (blue-green algae). *Toxicon* 26: 971–973.

CARMICHAEL WW, MAHMOOD NA, and HYDE EG. 1990. Marine toxins: origins, structure and molecular pharmacology. In: Hall S, Strichartz G [eds.], *Natural toxins from cyanobacteria (blue-green) algae*, 87–106. American Chemical Society, Washington, DC.

CARMICHAEL WW, DRAPEAU C, and ANDERSON DM. 2000. Harvesting of *Aphanizomenon flos-aquae* Ralfs ex Born. & Flah. var. *flos-aquae* (Cyanobacteria) from Klamath Lake for human dietary use. *Journal of Applied Phycology* 12: 585–595.

CARPENTER EJ, CAPONE DG, and REUTER JG [eds.]. 1992. *Marine pelagic cyanobacteria: Trichodesmium* and other *Diazotrophs. NATO ASI Series C, Mathematical and Physical Science.* Kluwer Academic Publishers, Dordrecht.

CASANOVA MT, BURCH MD, BROCK MA, and BOND PM. 1999. Does toxic *Microcystis aeruginosa* affect aquatic plant establishment. *Environmental Toxicology and Water Quality* 14: 97–109.

CHORUS I, and BARTRAM J [eds.]. 1999. *Toxic cyanobacteria in water. A Guide to their public health consequences, monitoring and management.* E&FN Spon, London.

CHORUS I, FALCONER IR, SALAS HJ, and BARTRAM J. 2000. Health caused by freshwater cyanobacteria in recreational water. *Journal of Toxicology and Environmental Health* 3: 323–347.

CODD GA. 1994. Biological aspect of cyanobacterial toxin. In: Steffensen DA, Nicholson BC, Adelaide SA [eds.], *Toxic cyanobacterial current, status research and management. Proceedings of the International Workshop*, 22–26 March. Australian Center for Water Treatment and Water Quality Research, Salisbury S.A.

CODD GA. 1995. Cyanobacterial toxins: occurrence, properties and biological significance. *Water Science and Technology* 32: 149–156

CODD GA. 2000. Cyanobacterial toxin, the perception of water quality, and the prioritisation if eutrophication control. *Ecological Engineering* 16: 51–60.

CODD GA, BELL SG and BROOKS WP. 1989. Cyanobacterial toxins in water. *Water Science and Technology* 21: 1–13.

DAVIDSON FF. 1959. Poisoning of wild and domestic animals by a toxic waterbloom of *Nostoc rivulare* Kuetz. *Journal of American Water Works Association* 51: 1277–1287.

DILLENBERG HO, and DEHNEL MK. 1959. Toxic water bloom in Saskatchewan. *Canadian Medical Association Journal* 83: 1151–1154.

DOW CS, and SWOBODA UK. 2000. Cyanotoxins. In: Wihtton BA, Potts M [eds.], *The ecology of Cyanobacteria*, 614–632. Kluwer Academic Publishers, Dordrecht.

DUY TN, LAM PKS, SHAW GR, and CONNELL DW. 2000. Toxicology and risk assessment of freshwater cyanobacterial (Blue-Green Algal) toxins in water. *Reviews of Environmental Contamination and Toxicology* 163: 113–186.

ERIKSSON JE. 1990. *Toxic peptides from Cyanobacteria – characterization and cellular mode of action*. Department of Biology, Abo Akademi University, Finland.

ERIKSSON JE, HAGERSTRAND H, and ISOMAA B. 1987. Cell selective cytotoxicity of peptide toxin from the cyanobacterium *Microcystis aeruginosa*. *Biochimica and Biophysica Acta* 930: 304–310.

ERIKSSON JE, MERILUOTO JAO, and LINDHOLM T. 1989a. Accumulation of peptide toxin from the cyanobacterium *Oscillatoria agardhii* in the freshwater mussel *Anadonta cygnea*. *Hydrobiologia* 183: 211–216.

ERIKSSON JE, PAATERO GIL, MERILUOTO JAO, and CODD GA. 1989b. Rapid microfilament reorganization induced in isolated rat hepatocytes by microcystin-LR, a cyclic peptide toxin. *Experimental Cell Research* 185: 86–100.

ERIKSSON JE, BRAUTIGAN DL, VALLEE R, OLMSTED J, FUJOKI H, and GOLDMAN RD. 1992. Cytoskeletal integrity in interphase cells require protein phosphatase activity. *Proceedings of the National Academy of Science, U.S.A.* 89: 11093–11097.

FALCONER IR. 1994a. Mechanism of toxicity of cyclic peptide toxins from blue-green algae. In: Falconer I [ed.], *Algal toxins in seafood and drinking water*, 177–187. Academic Press, Cambridge.

FALCONER IR. 1994b. *Detection methods for cyanobacterial toxins*. The Royal Society for Chemistry, Cambridge.

FALCONER IR. 1999. An overview of problems caused by toxic Blue-Green Algae (Cyanobacteria) in drinking and recreational water. *Environmental Toxicology and Water Quality* 14: 5–12.

FALCONER IR. 2001. Toxic cyanobacterial bloom problems in Australian waters: risks and impacts on human health. *Phycologia* 40: 228–233.

FALCONER IR, SMITH JV, JACKSON AR, JONES A, and RUNNEGAR MT. 1988. Oral toxicity of a bloom of the cyanobacterium *Microcystis aeruginosa* administered to mice over periods up to 1 year. *Journal of Toxicology and Environmental Health* 24: 291–305.

FLADMARK KE, SERRES MH, LARSEN NL, YASUMOTO T, AUNE T, and DOSKELAND SO. 1998. Sensitive detection of apoptogenic toxins in suspension cultures of rat and salmon hepatocytes. *Toxicon* 36: 1101–1114.

FLADMARK KE, BRUSTUGUN OT, HOVALD R, BOE R, GJERTSEN BT, ZHIVOTOVSKY B, and DOSKELAND SO. 1999. Ultrarapid scapase-3 dependent apoptosis induction by serine/threonine phosphatase inhibitors. *Cell Death and Differentiation* 6: 1099–1108.

FRANCIS G. 1878. Poisonous Australian lakes. *Nature* 18: 11–12.

FUJIKI H, and SUGANUMA M. 1993. Tumor promotion by inhibitors of protein phosphatase 1 and 2A: The okadaic acid class of compounds. *Advances in Cancer Research* 61: 143–196.

FUJIKI H, SUGANUMA M, SUGURI H, YOSHIZAWA S, TAKAGI K, NAKAYASU M, OJIKA M, YAMADA K, YASUMOTO T, MOORE RE, and SUGIMURA T. 1990. New tumor promoters from marine naturel products. In: Hall S, Strichartz G [eds.], *Marine toxins, origin, structure and molecular pharmacology*, 234–240. American Chemical Society, Washington DC.

GOLDBERG J, HUANG H-B, KWON Y-G, GREENGARD P, NARIN AC, and KURIYAN J. 1995. Three-dimensional structure of the catalytic subunit of protein serine/threonine phosphatase-1. *Nature* 376: 745–753.

GORHAM PR, and CARMICHAEL WW. 1988. Hazards of freshwater blue-green algae (cyanobacteria). In: Lembi CA, Waaland JR [eds.], *Algae and human affairs* 403–431. Cambridge University Press, Cambridge.

GUNN GJ, REFFERTY AG, REFFERTY GC, COCKBURN N, EDWARDS C, BEATTIE KA, and CODD GA. 1992. Fatal canine neurotoxicosis attributed to blue-green algae (cyanobacteria). *Veterinary Record* 4: 301–302.

HARADA KI. 1994. Strategy for trace analysis of microcystins in complicated matrix. *Proceeding of the Symposium: Toxic Cyanobacteria – A Global Perspective*, 49–51. Adelaide, South Australia: Australian Centre for Water Quality Research.

HART J, FAWELL JK, and CROLL B. 1997. The fate of both intra and extracellular toxins during drinking water treatment. Special subject No. 18, SS18-1-6, *IWSA World Congress*, Blackwell Science, Oxford.

HAWKINS PR, RUNNEGARD MTC, JACKSON ARB, and FALCONER IR. 1985. Severe hepatotoxicity caused by the tropical cyanobacterium (blue-green algae) *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Raju isolated from a domestic water supply reservoir. *Journal* of *Applied Environmental Microbiology* 50: 1292–1295.

HAWKINS PR, CHANDRASENA NR, JONES GJ, HUMPAGE AR, and FALCONER IR. 1997. Isolation and toxicity of *Cylindrospermopsis raciborskii* from an ornamental lake. *Toxicon* 35: 341–346.

HRUDEY S, BURCH M, DRIKAS M, and GREGORY R. 1999. Remedial measures. In: Chorus I and Bartram J. [eds.], *Toxic cyanobacteria in water. A guide to their public health consequences, monitoring and management*, 275–312. E&FN SPON, London.

HUBER CS. 1972. The crystal structure and absolute configuration of 2,9-diacetyl-9-azabicyclo[4,2,1] non-2,3-ene. *Acta Crystollogica* 238: 2577–2582.

HUMPAGE AR, and FALCONER IR. 1999. Microcystin-LR and liver tumor promotion: Effects on cytokinesis, ploidy, and apoptosis in cultured hepatocytes. *Environmental Toxicology and Water Quality* 14: 61–75.

JOCHIMSEN EM, CARMICHAEL WW, AN JS, CARDO DM, COOKSON ST, and HOLMES CEM. 1998. Liver failure and death after exposure to microcystins at a hemodialysis center in Brazil. *New England Journal of Medicine* 338: 873–878.

KEIJOLA AM, HIMBERG K, ESALA AL, SIVONEN K, and HIISVIRTA L. 1988. Removal of cyanobacterial toxins in water treatment processes: laboratory and pilot-scale experiment. *Toxicity Assessment* 27: 433–440.

LAHTI K. 1997. Cyanobacterial hepatotoxins and drinking water supplies-aspect of monitoring and potential health risks. In: *Monographs of boreal environment research,* no. 4. Finnish Environment Institute, Finland.

MACKINTOSH C, BEATTIE KA, KLUMPP S, COHEN P, and CODD GA. 1990. Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants. *FEBS Letter* 264: 187–192.

MAHMOOD NA, and CARMICHAEL WW. 1986. Paralytic selfish poisons produced by the fresh-water cyanobacterium *Aphanizomenon flos-aque* NH-5. *Toxicon* 24: 175–186.

MANKIEWICZ J, WALTER Z, TARCZYŃSKA M, ZALEWSKI M, FLADMARK KE, and DOSKELAND SO. 2000. Apoptotic effect of cyanobacterial blooms collected from water reservoirs. *Polish Journal of Occupational Medicine and Environmental Health* 13: 335–344.

MANKIEWICZ J, WALTER Z, TARCZYŃSKA M, FLADMARK KE, DOSKELAND SO, and ZALEWSKI M. 2001. Apoptotic effect of cyanobacterial extract on rat hepatocytes and human lymphocytes. *Environmental Toxicology and Water Quality* 3: 225–233.

MATSUNAGA S, MOORE RE, NIEMCZURA WP, and CARMICHAEL WW. 1989. Anatoxin-a(s), a potent anticholinesterase from *Anabaena flos-aquae. Journal of American Chemical Society* 111: 8021–8023.

MCDERMOTT CM, NHO CW, HOWARD W, and HOLTON B. 1998. The cyanobacterial toxin, microcystin-LR, can induce apoptosis in a variety of cell types. *Toxicon* 36: 1981–1996.

MCELHINEY J, LAWTON LA, and LEIFERT C. 2001. Investigations into the inhibitory effects of microcystins on plant growth, and the toxicity of plant tissues following exposure. *Toxicon* 39: 1411–1420.

MILLS JC, STONE NL, ERHARDT J, and PITTMAN RN. 1998. Apoptotic membrane blebbing is regulated by myosin light chain phosphorylation. *Journal of Cell Biology* 160: 627–636.

MUR L, SKULBERG O, and UTKILEN H. 1999. Cyanobacteria in the environment. In: Chorus I, Bartram J [eds.], *Toxic cyanobacteria in water: A guide to public health significance, consequences, monitoring and management,* 15–40. E&FN SPON, London.

MYNDERSE JS, MOORE RE, KASHIWAGI M and NORTON TR. 1977. Antileukemic activity in the Oscillatoriaceae, isolation of debromoaplysiatoxin from *Lyngbya. Science* 196: 538-540.

NICHOLSON BC, ROSITANO J, and BURCH MD. 1994. Destruction of cyanobacterial peptide hepatotoxins by chlorine and chloramine. *Water Research* 28: 1297–1303.

OHTA T, SUEOKA E, IIDA N, KOMORI A, SUGANUMA M, NISHIWAKI R, TATEMATSU M, KIM SJ, CARMICHAEL WW, and FUJIKI H. 1994. Nodularin a potent inhibitor of protein phosphatases 1 and 2A, is a new environmental carcinogen in male F344 rat liver. *Cancer Research* 54: 6402–6406.

OHTANI I, and MOORE RE. 1992. Cylindrospermopsin: a potent hepatotoxin from the blue-green algae *Cylindrospermopsis raciborskii. Journal of American Chemical Society* 114: 7941–7942.

PFLUGMACHER S, WIEGAND C, BEATTIE KA, CODD GA, and STEINBERG CEW. 1998. Uptake of the cyanobacterial hepatotoxin microcystin-LR by aquatic macrophytes. *Journal of Applied Botany* 72: 228–232.

PILOTTO LS, BURCH MD, DOUGLAS RM, CAMERON S, ROACH GJ, COWIE CT, BEERS M, ROBINSON P, KIRK M, HARDIMAN S, MOORE C, and ATTEWELL RG. 1997. Health effect of recreational exposure to cyanobacteria (blue-green algae) during recreational water activities. *Australian and New Zealand Journal of Public Health* 21: 562–566.

PREMAZZI G and VOLTERRA L. 1993. *Microphyte toxins. A manual for toxin detection, environmental monitoring and therapies to counteract intoxications.* JRC CEC, Luxembourg.

PREPAS EE, KOTAK BG, CAMPBELL LM, EVANS JC, HRUDNEY S.A., and HOLMES CFB. 1997. Accumulation and elimination of cyanobacterial hepatotoxins by the freshwater clam *Anodonta grandis simpsoniana. Canadian Journal of Fisheries Aquatic Sciences* 54: 41–46.

RABERGH CML, BYLUND G, and ERIKSSON JE. 1991. Histopathological effects of microcystin-LR, a cyclic peptide toxin from the cyanobacterium (blue-green algae) *Microcystis aeruginosa,* common carp (*Cyprinus carpio* L.). *Aquatic Toxicology* 20: 131-146.

REPAVICH WM, SONZOGNI WC, STANDRIDGE JH, WEDEPOHL RE, and MEISNER LF. 1990. Cyanobacteria (blue-green algae) in Wisconsin water acute and chronic toxicity. *Water Research* 24: 225–231.

RESSOM R, SAN SOONG F, FITZGERALD J, TURCZYNOWICZ L, EL SAADI O, RODER D, MAYNARD T, and FALCONER I. 1994. *Health effects of toxic Cyanobacteria (Blue – Green Algae)* 27–69. Australian Government Publishing Service, Canberra.

REYNOLDS CS. 1987. Cyanobacterial waterblooms. In: Callow P [ed.], *Advances in botanical research,* 17-143. Academic Press, London.

RINEHART KL, HARADA KI, NAMIKOSHI M, CHEN C, HARVIS CA, MUNRO MHG, BLUNT JW, MULLIGAN PE, BEASLEY VR, DAHLEM AM, and CARMICHAEL WW. 1988. Nodularin, microcystin, and the configuration of Adda. *Journal of American Chemical Society* 110: 8557–8558.

ROSITANO J, NICHOLSON BC, and PIERONNE P. 1996. Destruction of cyanobacterial toxins by ozone. *Proceedings of the First Australasian Conference of the International Ozone Association,* Sydney, Australia.

RUNNEGAR MT, KONG S, and BERNDT N. 1993. Protein phosphatase inhibition and *in vivo* hepatotoxicity of microcystins. *American Journal of Physiology* 265: G224–G230.

SINGH DP, TYAGI MB, and KUMAR A. 1999. Cyanobacterial toxins. In: Fatma T [ed.], *Cyanobacterial and algal metabolism and environmental biotechnology,* 61–72. Narosa Publishing House, New Delhi, India.

SIPIA VO, KANKAANPAA HT, FLINKMAN J, LAHTI K, and MERILUOTO JA. 2001. Time-dependent accumulation of cyanobacterial hepatotoxins in flounders (*Platichthys flesus*) and mussels (*Mytilus edulis*) from the northern Baltic Sea. *Environmental Toxicology and Water Quality* 16: 330–336.

SIVONEN K. 1996. Cyanobacterial toxins and toxin production. *Phycologia* 35: 12–24.

SIVONEN K, and JONES J. 1999. Cyanobacterial toxins. In: Chorus I, Bartram J [eds.], *Toxic cyanobacteria in water: A guide to*

public health significance, consequences, monitoring and management, 41–111. E&FN SPON, London.

SIVONEN K, HIMBERG K, LUUKKAINEN R, NIEMELA SI, POON GK, and COOD GA. 1989. Preliminary characterization of neurotoxic cyanobacteria blooms and strains from Finland. *Toxicity Assessment* 4: 339–352.

SKULBERG OM, CARMICHAEL WW, ANDERSON RA, MATSUNAGA S, MOORE RE, and SKULBERG R. 1992. Investigations of a neurotoxic oscillatorialean strain (Cyanophyceae) and its toxin. Isolation and characterization of homoanatoxin-a. *Environmental Toxicology and Chemistry* 11: 321–329.

SPIVAK CE, WITKOP B, and ALBUQUERQUE EX. 1980. Anatoxin-a: a novel, potent agonist at the nicotinic receptor. *Molecular Pharmacology* 18: 384–394.

STOTTS RR, NAMIKOSHI M, HASCHEK WM, RINEHART KL, CARMICHAEL WW, DAHLEM AM, and BEASLEY V. 1993. Structural modifications imparting reduced toxicity in microcystins from *Microcystis* spp. *Toxicon* 31: 783–789.

TARCZYŃSKA M, OSIECKA R, KONTEK R, BLASZCZYK A, and ZALEWSKI M. 1997. Przyczyny i konsekwencje toksycznych zakwitów sinicowych w zbiorniku. *Zeszyty Naukowe Komitetu "Człowiek i Środowisko"* 18: 59–74.

TARCZYŃSKA M, ROMANOWSKA-DUDA Z, JURCZAK T, and ZALEWSKI M. 2001. Toxic cyanobacterial blooms in a drinking water reservoirs – causes, consequences and management strategy. *Water Science and Technology* 1: 237–246.

TISDALE ES. 1931. Epidemic of intestinal disorders in Charleston, W. Va., occurring simultaneously with unprecedented water supply conditions. *American Journal of Public Health* 21: 198–200.

TOIVOLA DM, and ERIKSSON JE 1999. Toxins affecting cell signalling and alteration of cytoskeletal structure. *Toxicology in Vitro* 13: 521–530.

TOIVOLA DM, ERIKSSON JE, and BRAUTIGAN DL. 1994. Identification of protein phosphatase 2A as the primary target for microcistin-LR in rat liver homogenates. *FEBS Letter* 344: 175–180.

TOIVOLA DM, GOLDMAN RD, GARROD DR, and ERIKSSON JE. 1997. Protein phosphatases maintain the organization and structural interaction of hepatic keratin intermediate filaments. *Journal of Cell Science* 110: 23–33.

TURNER PC, GAMMIE AJ, HOLLINRAKE A, and COOD GA. 1990. Pneumonia associated with cyanobacteria. *British Medical Journal* 300: 1440–1441.

VASCONCELOS VM. 1995. Uptake and depuration of the heptapeptide toxin microcystin-LR in *Mytilus galloprovincialis*. *Aquatic Toxicology* 32: 227–237.

WHO. 1997. *Report of the Working Group Meeting on Chemical Substances in Drinking Water.* WHO, Geneva.

YU SZ. 1995. Primary prevention of hepatocellular carcinoma. *Journal of Gastroenterology and Hepatology* 10: 674–682.