Cyanobacterial Toxins: Removal during Drinking Water Treatment, and Human Risk Assessment

Bettina C. Hitzfeld, Stefan J. Höger, and Daniel R. Dietrich

Environmental Toxicology, University of Konstanz, Konstanz, Germany

Cyanobacteria (blue-green algae) produce toxins that may present a hazard for drinking water safety. These toxins (microcystins, nodularins, saxitoxins, anatoxin-a, anatoxin-a(s), cylindrospermopsin) are structurally diverse and their effects range from liver damage, including liver cancer, to neurotoxicity. The occurrence of cyanobacteria and their toxins in water bodies used for the production of drinking water poses a technical challenge for water utility managers. With respect to their removal in water treatment procedures, of the more than 60 microcystin congeners, microcystin-LR (L, L-leucine; R, L-arginine) is the best studied cyanobacterial toxin, whereas information for the other toxins is largely lacking. In response to the growing concern about nonlethal acute and chronic effects of microcystins, the World Health Organization has recently set a new provisional guideline value for microcystin-LR of 1.0 µg/L drinking water. This will lead to further efforts by water suppliers to develop effective treatment procedures to remove these toxins. Of the water treatment procedures discussed in this review, chlorination, possibly micro-/ultrafiltration, but especially ozonation are the most effective in destroying cyanobacteria and in removing microcystins. However, these treatments may not be sufficient during bloom situations or when a high organic load is present, and toxin levels should therefore be monitored during the water treatment process. In order to perform an adequate human risk assessment of microcystin exposure via drinking water, the issue of water treatment byproducts will have to be addressed in the future. *Key words*: cyanobacteria, ozone, risk assessment, toxin, water treatment. — *Environ Health Perspect* 108(suppl 1):113–122 (2000).
http://ehpnet1.niehs.nih.gov/docs/2000/suppl-1/113-122hitzfeld/abstract.html

Toxic blue-green algae in water used as drinking water or for recreational purposes poses a hazard to humans but has long been neglected or at most been treated on a local level. Scums of blue-green algae or cyanobacteria accumulating along the shores of ponds and lakes also present a hazard to wild and domestic animals. Providing the human population with safe drinking water is one of the most important issues in public health and will gain more importance in the coming millennium. Reports of toxic blooms and poisonings of humans and cattle range from the first report of a toxic *Nodularia* bloom in Lake Alexandrina, Australia, in 1878 (*1*), to a high incidence of primary liver cancer (PLC) in China attributed to cyanobacterial toxin-contaminated drinking water (*2–4*), to the recent tragic deaths of 60 dialysis patients in Caruaru, Brazil, in 1996 due to the presence of cyanobacterial toxins in the water supply used in a hemodialysis unit (*5,6*). The presence of cyanobacterial toxins in drinking water supplies poses a serious problem to water treatment facilities, since not all technical procedures are able to effectively remove these toxins to below acceptable levels. Despite this, it is highly unlikely that lethal poisonings would occur following consumption of drinking water contaminated with cyanobacterial toxins. Of much higher concern are low-level chronic exposures, since the risks associated with long-term exposure have not been adequately described. Drinking water suppliers are nevertheless confronted with a variety of questions ranging from what levels actually occur in the drinking water sources to the current state of knowledge about acute and chronic effects and effective water treatment technologies in removing toxins (*7*). This review addresses these issues.

Cyanobacteria

Morphology and Taxonomy

Cyanobacteria are an ancient group of organisms whose habitats range from hot springs to temporarily frozen ponds in Antarctica (*8*). They occur both in freshwater and in marine environments. Cyanobacteria, like eubacteria, lack a nucleus, whereas in contrast to their closest relatives, the purple and the green sulfur bacteria, they produce oxygen (*9*). According to the current taxonomy, 150 genera with about 2,000 species, at least 40 of which are known to be toxicogenic, have been identified (*10*). Cyanobacteria grow as single cells, as single cells in colonies, or as single cells in filaments, whereas some filamentous genera contain special nitrogen-fixing heterocysts. Cells growing in colonies may be packed in a mucilaginous sheath like *Microcystis* sp. or, in the case of filamentous species, grow as floating mats or as free-floating strands. Many cyanobacterial species possess gas vacuoles that allow them to regulate their position in the water column and give them a distinct ecologic advantage over other planktonic species.

Bloom Formation

Prevention of bloom formation is the most efficient method for avoiding cyanobacterial toxin contamination of drinking water. Unfortunately the factors leading to cyanobacterial bloom development (cell numbers > 10^6/L), whether of toxic or nontoxic species, have not been satisfactorily identified. Factors such as nitrogen, phosphorus, temperature, light, micronutrients (iron, molybdenum), pH and alkalinity, buoyancy, hydrologic and meteorologic conditions, and the morphology of the impoundment have all been implicated [for a discussion see Chorus and Bartram (*11*)]. More importantly, factors influencing toxin production have not been conclusively elucidated (*12*). Although these factors can be considered closely related to bloom formation, cell numbers and toxin levels are usually not closely related. Furthermore, few generalizations can be made from the few laboratory studies that have been conducted to date (*7,13–16*).

Cyanobacterial Toxins

Cyanobacteria produce a variety of toxins, subsequently called cyanotoxins, that are classified functionally into hepato-, neuro-, and cytotoxins. Additionally, cyanobacteria produce lipopolysaccharides (LPS) as well as secondary metabolites that are potentially pharmacologically useful. The former are responsible for the irritant nature of cyanobacterial material. Defined by their chemical structure, cyanotoxins fall into three groups: cyclic peptides (the hepatotoxins microcystins and nodularin), alkaloids (the neurotoxins anatoxin and saxitoxins), and LPS. The species most often implicated with toxicity are *Microcystis aeruginosa*, *Planktothrix* (= *Oscillatoria*) *rubescens*, *Aphanizomenon flos-aquae*, *Anabaena flos-aquae*, *Planktothrix agardhii*, and *Lyngbia* spp. (Table 1).

Address correspondence to B.C. Hitzfeld, PO Box X918, D-78457 Konstanz, Germany. Telephone: 49 7531 88 4105. Fax: 49 7531 88 3170. E-mail: bettina.hitzfeld@uni-konstanz.de

This work was partly supported by the Swiss Federal Office of Public Health, grant FE 316-98-0715. We also thank the Zurich Water Works, Zurich, Switzerland, for logistical support.

Received 20 July 1999; accepted 1 September 1999.

Table 1. Toxicity of cyanobacterial toxins.

Toxin	LD_{50} (µg/kg, ip, mouse)	Organism	Reference
Microcystin-LR	50	*M. aeruginosa, Aph. flos-aquae, M. viridis*	(*31,125*)
Microcystin-LA	50	*M. aeruginosa, M. viridis*	(*138*)
Microcystin-YR	70	*M. aeruginosa, M. viridis*	(*31*)
Microcystin-RR	600	*M. aeruginosa, Anabaena* sp., *M. viridis*	(*139–141*)
[*D*-Asp^3]microcystin-LR	50–300	*M. aeruginosa, Aph. flos-aquae, M. viridis, O. agardhii*	(*142,143*)
[*D*-Asp^3]microcystin-RR	250	*O. agardhii, M. aeruginosa, Anabaena* sp.	(*19,139*)
[*D*ha^7]microcystin-LR	250	*M. aeruginosa, Anabaena* sp., *O. agardhii*	(*139,144*)
[(6Z)-Adda]microcystin-LR	> 1200	*M. viridis*	(*143*)
[(6Z)-Adda]microcystin-RR	> 1200	*M. viridis*	(*143*)
Nodularin	50	*N. spumigena*	(*145*)
[*D*-Asp^1]nodularin	75	*N. spumigena*	(*146*)
[(6Z)-Adda^3]nodularin	> 2000	*N. spumigena*	(*146*)
Anatoxin-a	200–250	*Aph. flos-aquae, Anabaena* spp., *Oscillatoria* sp., *Aphanizomenon* sp., *Cylindrospermum* sp.	(*145,147*)
Anatoxin-a(s)	20	*Aph. flos-aquae*	(*148*)
Saxitoxin	10	*Aph. flos-aquae, A. circinalis, Cylindrospermopsis raciborskii, Lyngbya wollei*	(*42,149*)
Cylindrospermopsin	2000	*C. raciborskii, Umezakia natans, Aph. ovalisporum*	(*150*)

Cyclic Peptides

Microcystins and nodularins are the most widespread cyanotoxins. They can be found in cyanobacterial blooms ranging from freshwater bodies to oceans. Microcystins have been described from the genera *Microcystis, Anabaena, Planktothrix, Nostoc,* and *Anabaenopsis,* whereas nodularin has been found only in *Nodularia* (*17–21*).

Toxin synthesis. Although the environmental conditions under which cyanobacteria produce toxins remain largely unknown, the way these toxins are synthesized is becoming clearer. Their small size, cyclic structure, and content of unusual amino acids indicate that these peptides are synthesized nonribosomally rather than on ribosomes (*22,23*). The enzymes involved in nonribosomal peptide synthesis, peptide synthetases, have highly conserved structures. The genes coding for these peptide synthetases are modular, each module containing information for a single peptide synthetase unit. Using two conserved sequence motifs of the adenylate-forming domain of peptide synthetases to search for homologous sequences in toxic and nontoxic strains of *M. aeruginosa,* it was found that only the toxic strain contains peptide synthesis gene sequences (*23*). The ability of a cyanobacterial strain to produce toxins may thus depend primarily on the possession of these genes and on their expression under certain environmental conditions. With the emergence of a molecular genetics-based taxonomy of cyanobacteria together with the development of polymerase chain reaction primers and DNA probes specific for toxic strains of cyanobacteria, these toxin-producing strains may be identified more rapidly in the future (*24–26*).

Structure and uptake. These cyclic peptides (Figure 1) are rather small molecules with a molecular weight ranging from 800–1,000 Da (*17,27*). Most congeners are hydrophilic and generally not able to penetrate vertebrate cell membranes and therefore require uptake via an adenosine triphosphate (ATP)-dependent transporter. One thus far unidentified multispecific organic anion transporter (or bile acid transporter) has been described as the carrier of these cyclic peptides in rat liver (*28–30*). As a result of this, toxicity of microcystins and nodularins is restricted to organs expressing the organic anion transporter on their cell membranes, such as the liver. The structure of the heptapeptide microcystin was first identified in 1982 from an isolate of *M. aeruginosa.* Meanwhile, about 60 congeners with the general structure cyclo-(D-alanine^1-X^2-D-MeAsp^3-Z^4-Adda^5-D-glutamate^6-Mdha^7) have been characterized (Table 1) (*17,27, 31–33*). X and Z are two variable L-amino acids, D-MeAsp is D-*erythro*-β-methylaspartic acid, and Mdha is *N*-methyldehydroalanine. Adda is an unusual amino acid and unique to cyanobacterial toxins: (2S, 3S, 8S, 9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid. Nodularin is a pentapeptide with the general structure cyclo-(D-MeAsp^1-L-arginine^2-Adda^3-D-glutamate^4-Mdhb^5). Mdhb is 2-(methylamino)-2-dehydrobutyric acid. The most common structural variants occur in positions 2 and 4, resulting in substitutions of the L-amino acids, and demethylation of amino acids at positions 3 and/or 7. The current nomenclature names the most common structural variation, i.e. microcystin-LR (L, L-leucine; R, L-arginine) or microcystin-LW (W, L-tryptophane) (*34*). 6Z-stereoisomers of Adda have been reported for nodularin and microcystin (Table 1). [(6Z)-Adda^5]nodularin, [(6Z)-Adda^5]microcystin-LR, and [(6Z)-Adda^5]microcystin-RR have all been reported to be nontoxic in the standard mouse bioassay, with LD_{50} (median lethal dose) values of > 1,200 µg/kg body weight (bw).

Cylindrospermopsin

Cylindrospermopsin (Figure 1) is a structurally distinct toxin that has been found in tropical and subtropical waters of Australia, where it causes problems in water supplies (*35*). This alkaloid cyto- and hepatotoxin is produced mainly by *Cylindrospermopsis raciborskii* but also by *Aph. ovalisporum* and *Umezakia natans.*

Neurotoxins

The neurotoxins (Figure 2) described in cyanobacteria can be classified into three distinct groups: *a*) anatoxin-a and homoanatoxin-a; *b*) anatoxin-a(s), which is structurally not related to anatoxin; and *c*) saxitoxins or paralytic shellfish poisons (PSPs). Anatoxin-a has been described in *A. flos-aquae* and other *Anabaena* spp., *Planktothrix* sp. (*Oscillatoria* sp.), *Aphanizomenon* sp., *Cylindrospermum* sp. and in small amounts even in *Microcystis* sp. (*33,36,37*). Anatoxin-a exerts its neurotoxic effect by mimicking acetylcholine with an LD_{50} of 200–250 µg/kg bw (*36*). Anatoxin-a(s) is the only naturally occurring organophosphate and has been isolated from *A. flos-aquae* and *A. lemmermannii* (*38*). It is a highly toxic compound with an LD_{50} of 20 µg/kg bw (mouse ip) (*39*). Saxitoxins are better known from marine dinoflagellates (red tide) where they are responsible for paralytic shellfish poisoning after consumption of contaminated shellfish. But saxitoxins, a group of carbamate alkaloid neurotoxins, have also been detected in relevant amounts in freshwater cyanobacteria such as *Aph. flos-aquae, A. circinalis, C. raciborskii,* and *Lyngbya wollei* (*40–42*).

Microcystins

Animal Toxicity

Since the first description in 1878 of a *Nodularia spumigena* bloom in Lake Alexandrina, Australia (*1*), numerous cases of animal poisonings have been reported (Table 2). Most commonly, deaths of farm animals drinking scums of cyanobacterially contaminated ponds or poisonings of dogs swimming in cyanobacterial scum have been described (*7*). Fish kills have been reported in conjunction with cyanobacterial blooms and have often resulted in significant economic losses (*43–47*). The liver is the major target organ for microcystin toxicity; it was shown to accumulate 20–70% of a radioactively labeled toxin dose (intravenous) (*48–54*). Studies in mice and pigs exposed to extracts of a toxic *M. aeruginosa* bloom demonstrated dose-dependent toxicity (*55,56*). Increased mortality, liver weight, and plasma alanine

aminotransferase levels were associated with loss of body weight. Neither other organ systems nor lactate dehydrogenase levels were affected. Death of the organism through intrahepatic hemorrhage and shock is rapid, occurring within about 3 hr in the case of mice. Pathologic and ultrastructural features commonly observed in the liver are centrilobular hepatic necrosis, destruction of sinusoidal endothelium, disruption of bile canalicular function, intrahepatic hemorrhage, loss of microvilli and bleb formation in hepatocytes, and hepatocyte necrosis (*29,57–61*).

Inhibition of Protein Phosphatases 1 and 2A

The toxicity of microcystins and nodularins is due to inhibition of the catalytic subunit of protein phosphatases 1 and 2A (PP1, PP2A) (*62–64*). In the case of microcystins it has been suggested that covalent binding to cysteine-273 and cysteine-266 on PP1 and PP2A, respectively, is responsible for this effect (*65,66*). PP1 and PP2A dephosphorylate phosphoseryl or phosphothreonyl proteins and their inhibition leads to hyperphosphorylation of cytoskeletal proteins resulting in the deformation of hepatocytes (*28,67–71*). It is not clear, however, if the covalent binding of the toxin to PP1 or PP2A is in fact responsible for the inactivation, since inactivation precedes covalent modification and nodularin does not bind covalently (*72*). Furthermore, it has been suggested that the Adda side chain and possibly the planar ring portion of the peptide are responsible for both recognizing and inhibiting protein phosphatases (*73,74*).

Tumor Promotion

The cyanobacterial cyclic peptides possess tumor-promoting activity (TPA) by a TPA-independent pathway (*75*). Cyanobacterial extracts or microcystin-LR in drinking water induce skin tumors in rats and mice after initiation with 7,12-dimethylbenz[*a*]anthrazene (*76,77*). Glutathione-*S*-transferase placental form positive foci were detected in livers of rats after ip injection of microcystin-LR or nodularin and initiation with diethylnitrosamine (*78–80*). It has been speculated that these toxins may be liver carcinogens, since they induce foci or small neoplastic nodules without the use of initiators (*80,81*). Both microcystin-LR and nodularin induce the expression of tumor necrosis factor-α and early response genes (c-*jun*, *jun* B, *jun* D, c-*fos*, *fos* B, *fra*-1) in rat liver and hepatocytes (*80,82*). In addition, mutations in the K-*ras* codon 12 in the RSa cell line (*83*) and DNA fragmentations have been reported after ip injection of cyanobacterial extract or microcystin-LR in mice (*84,85*). These *in vitro* and

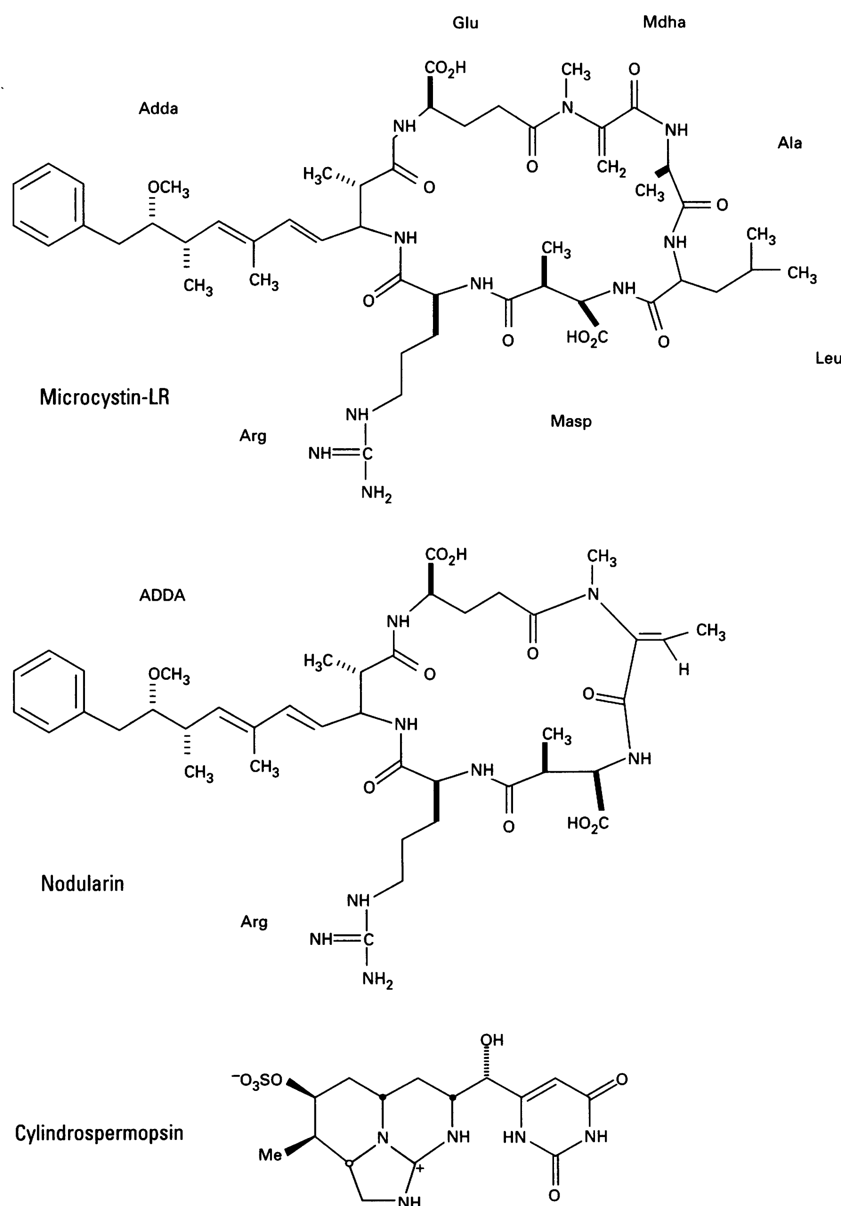

Figure 1. Structure of microcystin, nodularin, and cylindrospermopsin.

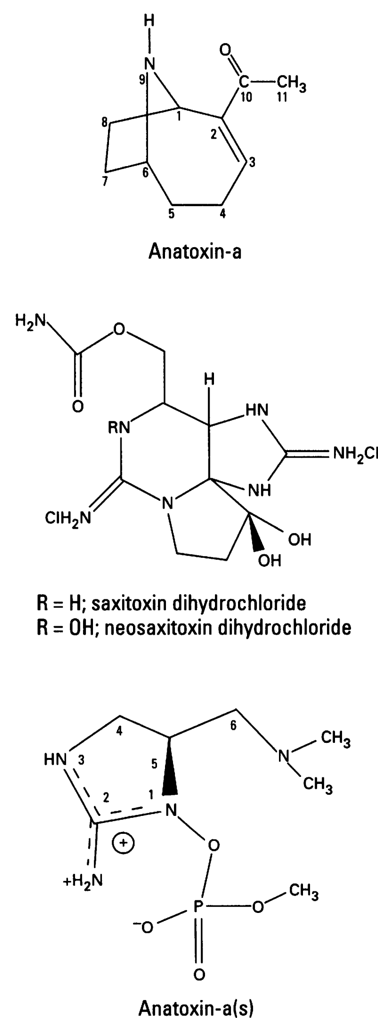

Figure 2. Structure of anatoxin-a, saxitoxins, and anatoxin-a(s).

Table 2. Toxic cyanobacteria episodes.

Location	Year	Case	Organism	Toxin	Reference
Lake Alexandrina, Australia	1878	Livestock stupor	*Nodularia spumigena*	Nodularin[a]	(*1*)
Ohio River, U.S.	1931	Humans: gastroenteritis	Unspecified cyanobacteria	Unknown	(*86*)
Harare, Zimbabwe	1966	Humans: gastroenteritis	*Microcystis aeruginosa*	Unknown	(*87*)
Alpine Lakes, Switzerland	1974–1994	Cattle deaths, liver damage	*Oscillatoria limosa, O. tenuis, Phormidium* sp., *Tychonema* sp., *Pseudoanabaena catenata, P. autumnale* a.o.	PPI-toxin[b]	(*151*)
Armidale, Australia	1983	Humans: liver damage	*Microcystis aeruginosa*	Unknown	(*88*)
Palm Island, Australia	1983	Humans: hepatoenteritis	*Cylindrospermopsis raciborskii*	Cylindrospermopsin	(*152*)
Richmond Lake, U.S.	1988	Livestock, birds, dogs, fish	*Anabaena flos-aquae, Aphanizomenon flos-aquae, Microcystis aeruginosa*	Anatoxin-a(s)	(*153*)
Rudyard Reservoir, U.K.	1989	Humans: recruits, pneumonia, diarrhea	*Microcystis aeruginosa*	Microcystin-LR	(*154*)
Darling River, Australia	1990–1991	Livestock deaths 1000-km bloom	*Anabaena circinalis*	Saxitoxin Neosaxitoxin	(*42*)
Loch Insh, Scotland, U.K.	1992	Dog death	*Oscillatoria* sp.	Anatoxin-a	(*155*)
Itaparica Dam, Brazil	1993	Humans: 88 deaths, gastroenteritis	*Anabaena* sp., *Microcystis* sp.	Unknown	(*89*)
Loch Leven, Scotland, U.K.	1994	Fish deaths	*Anabaena flos-aquae*	Microcystin	(*44*)
Nandong District, Jiangsu Province, Nanhui/Shanghai, Fusui, China	1994–1995	Humans: PLC 100 of 10^5 toxins act with HBsAg and aflatoxin	*M. aeruginosa, Planktothrix agardhii, Anabaena* sp., *O. tenuis, Lyngbya* sp.	Microcystins	(*156*) (*2–4*)
Caruaru, Brazil	1996	Hemodialysis patients: 60 deaths	*Aphanizomenon* sp., *Oscillatoria* sp.	Microcystins	(*5,6*)

Abbreviations: HBsAg, hepatitis B antigen; PLC, primary liver cancer; PPI, protein phosphatase inhibition. [a]Suspected. [b]Protein phosphatase-inhibiting activity.

in vivo data have to be viewed in the light of observations in China, where consumption of microcystin-contaminated drinking water has been associated with a high incidence of PLC (*2–4*) (see next section).

Human Health Effects

Evidence of human poisonings by cyanobacterial toxins ranges from health effects after recreational exposure to poisonings following consumption of contaminated drinking water (Table 2).

Acute and subchronic exposures. The earliest case of gastroenteritis from cyanobacteria was reported in 1931 in towns along the Ohio River, where low rainfall had caused the development of a large cyanobacterial bloom (*86*). The water treatment procedures employed over months to combat this bloom (prechlorination, sedimentation, filtration, chlorination, copper sulfate to lyse the cyanobacterial cells, aeration, activated carbon, permanganate, ammonia, and dechlorination) all proved to be ineffective in reducing taste, odor, or toxin content of the drinking water. A natural *Microcystis* bloom in a water reservoir in Harare, Zimbabwe, caused gastroenteritis in children each year when the bloom was decaying (*87*). A particularly extensive and toxic (microcystin-YM [Y, L-tyrosine; M, L-methionine]) (*31*) *M. aeruginosa* bloom in Malpas Dam, near Armidale, Australia, was treated with copper sulfate in 1981 after complaints of bad taste in the drinking water were received (*88*). The plant treating the water used prechlorination, alum flocculation, sedimentation, rapid sand filtration, postchlorination, and fluoridation. The effect of this toxic bloom event was then monitored in a retrospective epidemiologic study of liver function in the population consuming the water. It was found that the serum level of the liver enzyme γ-glutamyltransferase was elevated in that part of the population using the Malpas Dam water during the bloom and after the bloom was lysed with copper sulfate. The two most lethal poisonings attributed to cyanobacteria in drinking water occurred in Brazil. A massive *Anabaena* and *Microcystis* bloom in Itaparica Dam was responsible for 2,000 gastroenteritis cases resulting in 88 deaths, mostly children (*89*). A very tragic though relatively well-documented case occurred in a hemodialysis center in Caruaru in 1996 (*5,6*). The water used in the dialysis unit, taken from Tabocas Reservoir, was normally sedimented with alum, filtered, and chlorinated in the municipal water treatment plant prior to being supplied by truck to the clinic. At the dialysis unit, the water was further purified by passing through sand, charcoal, and an ion-exchange resin, and finally by micropore filtration. During the 1996 summer drought, the dialysis center received water from the municipal plant that was only treated with alum, but not filtered or chlorinated. There are conflicting reports as to whether the water was chlorinated in the trucks prior to delivery to the clinic. Furthermore, the charcoal, sand, and micropore filters at the clinic had not been changed in the 3 months prior to this episode, even though the water received from the trucks had been visibly turbid. In February 1996, the majority (85%) of the hemodialysis patients developed a toxic illness of varying severity, with a wide range of neurologic symptoms as well as acute liver injury. Up to 23 patients died in the first 2 weeks of this episode with either neurologic symptoms or from liver failure. About 37 more patients died in the following 5 weeks either directly from hepatotoxic effects or from complications such as sepsis, gastrointestinal bleeding, or cardiovascular effects. The patients displayed cholestatic jaundice with high bilirubin and alkaline phosphatase concentrations, and increases in hepatic enzymes (aspartate and alanine aminotransferase). Liver pathology showed the presence of an acute novel toxic hepatitis similar to that seen in animals exposed to microcystins (*6*). Histopathology showed panlobular hepatocyte necrosis, together with cell-plate disruption and apoptosis. However, in contrast to animal models of microcystin intoxication, no intrahepatic hemorrhage could be observed. After initial uncertainties as to the causative agents of these fatal intoxications, microcystin concentrations were determined in serum and in liver tissue as well as in the water filtration columns. The latter contained intact and fragmented cyanobacterial cells as well as microcystin-LR. Levels in serum ranged from 1 to 10 ng/mL; concentrations in the liver were as high as 0.6 mg/kg tissue. Toxin congeners were reported to be microcystin-YR, microcystin-LR, and microcystin-AR (A, L-alanine). At the time of the outbreak, cyanobacterial counts had not been made in the reservoir, but in March 1996, it was found that the most common cyanobacterial genera present were *Aphanizomenon, Oscillatoria,* and *Spirulina.*

Chronic exposure. When considering the chronic effects of long-term exposure to microcystins in drinking water, one has to take into account the high incidences of PLC in regions of China where pond and ditch water are used as drinking water supplies. In Haimen and Qidong (Jiangsu Province), pond and ditch water used as drinking water showed average microcystin concentrations of 160 pg/mL (60% of the samples analyzed were positive), whereas microcystins could not be detected in well water (*2–4*). PLC

incidences of 4.28 per 100,000 and 100.13 per 100,000 were observed in humans using well water and pond/ditch water, respectively (*2*). It has been calculated that humans living in areas with a reported high PLC incidence consume 0.19 pg microcystin per day during the 4 summer months from June to September over their 40- to 50-year life span (*4*). Coexposure to the potent liver carcinogen aflatoxin B_1 or to hepatitis B virus may result in the high incidence of PLC in this region (*4*).

Efficacy of Water Treatment Procedures

Water treatment measures should always be just one option after other techniques such as selection of intake depth, offtake by bank filtration, and/or use of barriers to restrict scum movement have been used. When evaluating water treatment procedures for the removal of cyanobacterial toxins, one is faced with problems regarding soluble and suspended substances. Cyanotoxins are produced within the cyanobacterial cells and thus toxin removal involves measures to destroy or avoid the cells. The cyanotoxins also are all water soluble, thus remediation measures involve chemical procedures reducing the toxicity or completely removing the toxins from the drinking water.

Most studies, especially early ones, had to rely on relatively crude measurements of acute toxicity, since more specific analytical methods were not available. The cyclic heptapeptides have been the focus of most of these studies, but some studies on removal of saxitoxins and anatoxin-a also exist.

Coagulation/Flocculation, Dissolved Air Flotation, and Activated Carbon Adsorption

Coagulation or flocculation involves the aggregation of smaller particles into larger particles using chemicals such as ferric chloride or aluminum sulfate. Coagulation can be an efficient method for eliminating cyanobacterial cells from water, whereas soluble cyanotoxins are not very efficiently removed by this method (*90,91*). The efficiency of cyanobacterial removal is dependent on an optimization of chemical doses and coagulation pH (*92*). Coagulation may cause additional problems such as lysing of cyanobacterial cells leading to release of toxins (*90*). When employing dissolved air flotation (DAF), it is important to consider that different cyanobacterial species behave differently depending on their physical properties: in a Belgian DAF plant *Microcystis* was removed by 40–80%, *Anabaena* by 90–100%, but *Planktothrix* only by 30% (*93*). Because conventional water treatment usually involves a combination of these methods, most of the research has focused on the effect of coagulation/flocculation in combination with other measures. In one of the earliest studies, toxins isolated from algal material were subjected to *a*) activated carbon filtration; *b*) prechlorination, flocculation with $FeCl_3$, sedimentation, sand filtration, and activated carbon filtration; and *c*) lime pretreatment, flocculation with $FeCl_3$, chlorination, and activated carbon filtration (*94*). The toxicity of these samples was then tested in the mouse bioassay. Chlorination, flocculation, or sand filtration were unable to destroy the toxins; only the last step, powdered activated carbon (PAC) at a ratio of 1:10 to 1:100 (toxin:activated carbon), removed a toxin concentration of 3 µg/mL to below toxic levels. Studies using 50 mg lyophilized cyanobacteria also show that conventional flocculation, filtration, and chlorination are not efficient in destroying the toxins: high performance liquid chromatography (HPLC) analysis shows a toxin reduction of up to only 34% (*95,96*). Only the inclusion of a treatment step with activated carbon resulted in 100% removal of the toxins from water. On the basis of the results of these laboratory studies, Lahti and Hiisvirta (*97*) conducted pilot-scale experiments to study the feasibility of predicting the behavior of cyanobacterial toxins in water treatment practice. Both fresh and freeze-dried cyanobacteria were subjected to the following processes: *a*) flocculation with $Al_2(SO_4)$ plus sedimentation plus filtration, and *b*) PAC plus flocculation with $Al_2(SO_4)$ plus sedimentation plus filtration. Toxicity was measured using the mouse bioassay and HPLC. As was to be expected from the laboratory scale studies, only the inclusion of PAC significantly reduced toxicity. Activated carbon is, however, not always a very efficient method. A study aimed at the removal of cyanobacterial cells with rapid sand filtration and activated carbon found a reduction of cyanobacteria of only 42% (*98*). More detailed studies with activated carbon show that both PAC as well as granular-activated carbon (GAC) effectively and quickly (contact times of 30 min are sufficient) eliminate cyanotoxins from water (*99–101*). In the case of PAC, dosing is an important parameter (10 µg/L toxin: > 200 mg/PAC/L), whereas when using GAC, the choice of the carbon source is important (coal, wood > peat, coconut), probably due to the different pore sizes relative to the size of the microcystin molecule (*102*). A major concern when using activated carbon in water treatment plants is the formation of a biofilm, which can significantly impair the ability of the filter to adsorb toxins; biodegradation by the biofilm does not seem to occur (*99,103,104*). Furthermore, below concentrations of 0.15 µg microcystin-LR/L, very little microcystin will be removed by activated carbon in the presence of a biofilm or natural organic matter (*103*). This finding has consequences for the risk assessment of a chronic exposure to low microcystin concentrations.

Rapid Filtration and Slow Sand Filtration

The performance of rapid filtration, a method usually employed after coagulation to remove the floc, does not effectively remove cyanobacterial cells (*98,105*). Conventional water treatment requires regular backwashing of the filters, but if this washing process is performed inadequately, lysis of cyanobacterial cells on the filters can lead to release of toxins into the water (*11,106*). Furthermore, sand filtration alone does not lead to substantial reduction of toxicity (*99*), and blocking caused by overloading should be avoided (*11*).

Chlorination

In general, chlorination is not an effective process in destroying cyanotoxins (*94–96, 107*). The efficiency of chlorination seems to depend largely on the chloride compounds and the concentration used. Aqueous chlorine and calcium hypochlorite at ≥ 1 mg/L remove more than 95% of microcystins or nodularin, while sodium hypochlorite at the same dose or chloramine achieve 40–80% removal at most (*91,108,109*). A chlorine residual of at least 0.5 mg/L should be present after 30 min contact time in order to destroy cyclic peptides completely (*110*). It should be noted, however, that even when acute toxicity, as measured by the mouse bioassay, was removed by this process, progressive liver damage could still be detected in the animals. This subacute toxicity may be due to incomplete toxin removal or to the formation of chlorination byproducts, which have been implicated in toxicity (*111*). Anatoxin-a or saxitoxins could neither be destroyed with chlorine doses exceeding a 30-min chlorine demand nor by changes in pH (*107,108*). Cylindrospermopsin, on the other hand, was effectively oxidized by 4 mg/L chlorine at pH 7.2–7.4 (toxin concentration 20–24 µg/L) (*108*).

Light

Microcystins are very stable under natural sunlight (*112*), whereas ultraviolet (UV) light around the absorption maxima of microcystin-LR and microcystin-RR rapidly decomposed the toxins (*113*). A photocatalytic process using a TiO_2 catalyst and UV radiation also quickly decomposed microcystin-LR, -YR, and ≠YA with half-lives of < 5 min (*114*). The efficiency of this process was largely dependent on the organic load of the water (*114*).

Membrane Processes

Microfiltration (MF) and ultrafiltration (UF) are technologies that have emerged in recent

years and have therefore not been thoroughly investigated as to their efficiency in removing cyanobacterial cells or toxins (*7*). One study showed that both UF and MF can be very efficient (> 98%) in removing whole cells of toxic *M. aeruginosa* (*115*). An important point when considering filtration is the lysis of cells. In the case of the above-cited study, some damage to cells could be observed, but toxin was not detected in the filtrate. UF was also effective in reducing microcystin and nodularin levels in the filtrate. This may be expected from a membrane with a very low-molecular-weight cut-off pore size (nanofiltration membrane) (*101,116*).

Ozonation

In Europe and North America, ozonation has been used primarily for disinfection purposes or to remove color and/or odor (*117*). Ozone was initially used at the beginning of the water treatment train mainly to inactivate viruses and bacteria. In recent years, though, many water treatment plants have included a two-stage ozonation treatment, either with pre- and interozonation, inter- and postozonation, or with pre- and postozonation.

In water, two pathways for the oxidation of organic pollutants by ozone have been described (*117,118*): direct attack by molecular ozone via cycloaddition or electrophilic reaction, and indirect attack by free radicals (primarily $^{\bullet}OH$) formed by the decomposition of ozone. The mechanism involving cycloaddition in water usually results in the formation of aldehydes, carboxylic acids, ketones, and/or carbon dioxide. The electrophilic attack by molecular ozone probably occurs on atoms carrying negative charge such as N, P, O, or nucleophilic C. An indirect attack by free radicals generally occurs via one of three pathways: hydrogen abstraction, electron transfer, or radical addition.

Microcystins and nodularin. Ozone is one of the most powerful oxidizing agents and its potential to destroy cyanobacterial toxins has been investigated in the last 10 years (Table 3).

In one of the earliest studies looking at the effect of ozone on cyanotoxins, researchers at the British Foundation for Water Research ozonated microcystin-LR purified from *M. aeruginosa* and assessed toxicity using a mouse bioassay (*90,119*). After ozonation, the toxicity of the cyanotoxin is reduced, which could be shown by a prolongation of mouse survival time, but the results cannot be quantified since the authors omitted to detail ozone or toxin concentrations. HPLC and fast atom bombardment-mass spectrometry analysis also show a reduction in the microcystin peak after a 2-sec ozonation. Interestingly, several new peaks appeared but were not tested separately for toxicity. This very fast destruction of microcystin was corroborated in Australian studies quantifying the effect of ozone on different microcystin-LR concentrations (*91,108,120*) and in our own work (*121*). These studies showed that up to 800 µg/L microcystin-LR can be oxidized to below the HPLC detection limit by < 0.2 mg/L ozone within seconds to minutes. The reaction of ozone with nodularin also occurs very rapidly: when reacting 88 µg/L nodularin with 0.05 mg/O_3, there was zero toxin recovery after 15 sec (*120*). With these studies it was also demonstrated that the removal of microcystins is proportional to the ozone dose when the microcystin concentration is below the ozone demand (*91*). Complete removal of microcystin is achieved and an ozone residual is detected when the ozone demand of the water has been met.

Cyanobacterial extracts and cells and organic load. Obviously, a more realistic way to test the efficiency of ozonation would be to use either cyanobacterial extracts or whole cells (Table 4). Oxidation reactions of ozone with cyanobacterial toxins are always in competition with other organic compounds in the water. As a result, naturally occurring organic matter is one of the most important factors to consider in terms of toxin dynamics. In a study designed to model continuous operation, ozone doses from 1 to 10 mg/L were tested over 5–10 min for their ability to degrade 10 µg/L microcystin added to different water sources (*101*). Two milligrams per liter ozone added to raw water leads to a 60% removal of microcystin, whereas the same dose added to treated water removes toxins by 98%. A similar ozone demand was measured in a study; 500 µg/L microcystin-LR was oxidized with 0.2 mg/L O_3 over 4 min in organic-free water (*122*). The author calculated an ozone demand of 0.6 mg/L with almost complete microcystin removal. However, only 50% of the same microcystin concentration was removed when filtered Seine River (France) water was oxidized with 0.5 mg/L O_3 over 10 min. This led to a much higher ozone demand of 1.6 mg/L. Our own results show that cyanobacterial extracts (*M. aeruginosa* or *P. rubescens*) containing 50–100 µg/L microcystin-LR–equivalents need to be oxidized with at least 1.0 mg O_3/L to effectively destroy the toxins present, whereas ozone residuals were undetectable after 10 min (*106*). These results show that ozone consumption by natural organic matter still occurs at the preozonation stage. During postozonation, 1 mg/L O_3 removed 38% of the microcystin, whereas > 2 mg/L O_3 removed toxin related toxicity below the limit of detection. The importance of organic load and ozone concentration was also demonstrated in Australian studies: cyanobacterial extracts containing 135–220 µg/L microcystin-LR required 1.0 mg/L ozone over 5 min for complete toxin destruction (*108,120*). After this treatment, the ozone residual was zero, reflecting the higher organic load and resultant high ozone demand. The critical importance of ozone dose, especially with respect to the organic load of the water, was also shown in several Finnish studies (*95–97*). Fresh and freeze-dried natural bloom material (*M. aeruginosa, M. wesenbergii, M. viridis*) from a Finnish lake (LD_{50} 60–75 mg/kg bw, mouse ip) as

Table 3. Effect of ozone on destruction of cyanobacterial toxins in the presence or absence of organic matter.

Microcystin-LR (µg/L)	Ozone dose (mg/L)	Duration (min)	OM present	Destruction (%)	Ozone demand (mg/L)	Ozone residual (mg/L)	Reference
21	1.2	5	–	73	ND	0.13	(*101*)
9	1.0	5	+	50	ND	0	(*101*)
500	0.2	4	–	99	0.6		(*122*)
500	0.5	10	+	50	1.6		(*122*)
≤ 200	1.0	5	–	100	ND	0	(*108,120*)
15	1.0–1.5	30	+	50	ND	ND	(*95–97*)
50	1.0–1.5	30	+	90	ND	ND	(*95–97*)
10	0.5–1.5	9	–	90–100	ND	0.4–1.2	(*121*)
50–100	0.5–1.5	9	+	0–100	ND	0.1–0.6	(*121*)

ND, not determined; OM, organic matter.

Table 4. Effect of ozone on destruction of cyanobacterial toxins from cells in the presence or absence of organic matter.

Microcystis aeruginosa (cells/mL)	Ozone dose (mg/L)	Duration (min)	Destruction (%)	Ozone demand (mg)	Ozone residual (mg/L)	Reference
1.63×10^6	3.7	5	36	ND	0	(*120*)
2.05×10^6	2.5	12	100	29	ND	(*120*)
1×10^4	0.8	10	60	ND	0.01	(*107*)
1×10^{5a}	1.3	10	65	ND	0	(*107*)
1×10^5	1.0–1.5	9	50–100	ND	0.25–1.4	(*106*)
5×10^5	1.0–1.5	9	30–75	ND	0.4–0.8	(*106*)

ND, not determined. [a]In raw water; all other experiments in pure or filtered water.

well as a laboratory culture of *P. agardhii* (NIVA-CYA 126; LD_{50} 190 mg/kg bw, mouse ip) were used. In a pilot plant setup, 35 mg/L fresh (50 µg/L toxin) or 24 mg/L (15 µg/L toxin) freeze-dried cyanobacteria were subjected to preozonation at a dose of 1.0–1.5 mg O_3/L. This treatment resulted in a reduction of toxicity by 50% (freeze-dried) and 90% (fresh). As can be seen, toxin reduction from fresh cyanobacteria was better than from freeze-dried material. This may be explained by the improved coagulation caused by preozonation. Preozonation has been widely used to assist coagulation (*117*). The major problem associated with this method is the danger of cell lysis and toxin release. A second postozonation step, using an ozone concentration high enough to oxidize the remaining organic matter and toxin, would then be essential. The experiment with freeze-dried material corresponds to a situation where the bloom disintegrates and cells lyse due to chemical treatment or as a result of natural causes. Preozonation with 0.5–1.0 mg O_3/L in that case is not the most effective treatment. Postozonation is a preferred method, since more of the oxidation capacity could be used on toxins instead of on other organic material. A common problem with these early studies is, however, that toxicity was determined by the mouse bioassay (detection in the microgram range) or by HPLC (detection in the nanogram–microgram range), two relatively insensitive assays (*123*).

Ozonation of intact cells during preozonation steps poses the risk of cyanobacterial lysis and increased ozone demand. If cyanobacteria are not monitored at the water intake level and thus enter the water treatment process, the treatment plant may not be prepared to meet the increased ozone demand. This leads to either an increase in the soluble toxin concentration in the water and/or to incomplete degradation of the cyanotoxins. Simulating bloom situations, studies were performed with *M. aeruginosa* concentrations from 1×10^4 to 2×10^6 cells/mL (*107,120*) (Table 4). Depending on cell number and organic load of the spiked water, an ozone demand between 2 and 3 mg/L (over 5 min) and 29 mg/L (over 12 min) was calculated. This ozone demand is relatively high considering that water treatment plants regularly employ a concentration of 0.5 mg/L at preozonation and 1.0 mg/L at postozonation stages, respectively. This can also be seen in our own study where ozonation with 1.0 mg/L O_3 did not completely destroy the toxins present in 1×10^5 cells/mL (*106*) (Table 4). Furthermore, when a culture of 1.63×10^6 *M. aeruginosa* cells/mL was ozonated with a maximum of 3.7 mg O_3/L, only 36% of total toxin was removed after 5 min (*120*).

Anatoxin-a, anatoxin-a(s), and saxitoxins. The efficiency of oxidation with ozone with respect to anatoxin-a, anatoxin-a(s), or the saxitoxins (PSPs) has not been well characterized (*95,97*). Using raw and filtered waters, the British Foundation for Water Research determined that anatoxin-a is more resistant to removal by ozone than microcystin-LR (*107*). The maximal ozone dose applied (4.5 mg/L) in raw water reduces the anatoxin-a concentration from 2.4 µg/L to 0.6 µg/L, whereas no ozone residual could be detected. In filtered water, without competition from natural organic material, 2.2 mg/L O_3 destroys an anatoxin-a concentration to below the limit of detection (0.3 µg/L). But again, no ozone residual could be detected. The PSPs require even higher O_3 doses. Ozonation over 15 min at 4.2 mg/min was necessary to reduce the neurotoxicity of an *A. circinalis* extract to near the lethal threshold concentration (*120*). After 30 min ozonation, the mice survived the doses. There are indications that other PSP toxins, such as GTX2, dcGTX2, dcGTX3, C1, and C2, may also be effectively oxidized by ozone (*110*). These studies stress the need for more detailed and quantifiable studies regarding the efficiency of ozone in destroying the neurotoxins.

pH. A very important parameter in the oxidation efficiency of ozone is pH. At pH values > 7.5, toxins can still be detected in the samples. This is due to the lower oxidation potential of ozone under alkaline conditions (1.24V) compared to acidic conditions (2.07V).

Ozonation byproducts. In contrast to chlorination byproducts, the issue of ozonation byproducts has not been properly addressed. One has to keep in mind though, that the amount of ozone applied is always less than what would be required to oxidize all the organic material to CO_2 and H_2O, especially in water high with organic content. One can therefore expect semioxidation products to form (*124*). Such oxidation products were found by HPLC when cyanobacteria were preozonated. Their toxicity was, however, not investigated (*97*). Our work points to ozonation products that still exhibit phosphatase inhibitory activity, but their structure has not yet been determined (*121*). An indication of the effect of ozone on microcystins stems from chemical characterization studies, since ozonolysis has been widely employed for structural characterization of organic compounds by cleavage of carbon–carbon double bonds (*125,126*). In the case of microcystins and nodularin, ozonolysis has been applied in the determination of the absolute configurations of the Adda moiety (*126*). It has been described that the double bond between C-6 and C-7 of the Adda side chain is easily cleaved by ozone to give 3-methoxy-2-methyl-4-phenylbutyric acid. In order to realistically assess the consequences of ozonation on cyanobacterial toxins, the ozonation byproducts have to be identified and their toxicity tested not only in acute tests but also in subacute tests such as the phosphatase inhibition assay as well as in chronic situations.

Risk Assessment

The health risk posed by exposure to cyanotoxins is difficult to quantify, since the actual exposure and resulting effects have still not been conclusively determined, especially for the human situation. The most likely route for human exposure is the oral route via drinking water (*127,128*), recreational use of lakes and rivers (*129*), or consumption of algal health food tablets (*130*). The dermal route may play a role during the recreational use of water bodies (swimming, canoeing, etc.) (*127,128*).

Due to the growing concern about health effects of cyanotoxins, especially via drinking water, the World Health Organization (WHO) has adopted a provisional guideline value for microcystin-LR of 1.0 µg/L in 1998 (*131*). This guideline value is based on a tolerable daily intake (TDI) value derived from two animal studies (*56,132*). The first study is a 13-week mouse oral study that determined a no-observable adverse effect level (NOAEL) of 40 µg/kg bw per day based on serum enzyme levels and liver histopathology (*132*). Applying a total uncertainty factor of 1,000 (10 for intra- and interspecies variability, respectively, and 10 for limitations in the database, especially lack of data on chronic toxicity and carcinogenicity), a provisional TDI of 0.04 µg/kg bw per day has been derived for microcystin-LR. This TDI was supported by a 44-day pig oral study that determined a lowest observable adverse effect level (LOAEL) of 100 µg microcystin-LR equivalents/kg bw per day (*56*). In this study, the cyanobacterial material fed to the pigs contained several microcystin congeners, but only microcystin-YR was tentatively identified. To this LOAEL a total uncertainty factor of 1,500 was applied (10 for intraspecies variability, 3 for interspecies variability, 5 for extrapolating from a LOAEL to a NOAEL, and 10 for the less-than-lifetime exposure). This resulted in a provisional TDI of 0.067 µg/kg bw per day. WHO used the lower of these two values for establishing the provisional guideline value. This value is calculated by applying the TDI (0.04 µg/kg bw) to a typical daily water intake in liters (L = 2 liters) by an individual of a given body weight (bw = 60 kg) and a proportion (P = 0.8) of the total daily intake to the intake by drinking water:

$$Guideline\ value = \frac{TDIxbwxP}{L}$$

The resulting value of 0.96 µg/L was rounded to 1.0 µg/L and should be applied to cyanobacterial cell-bound and extracellular

microcystins. This provisional guideline value is applicable only for microcystin-LR, since the database for other microcystin congeners or even other cyanotoxins such as the saxitoxins is too small to derive a TDI. Health Canada is applying an uncertainty factor of 3,000 to the NOAEL of 40 µg/kg bw per day from the 13-week mouse study by adding a factor of 3 for evidence of tumor promotion and weak evidence of a potential for carcinogenicity in humans (*133*). They thus derive a TDI of 0.013 µg/kg bw per day and conclude that the consumption of 1.5 L drinking water containing < 0.5 µg microcystin-LR/L by a 60-kg person would not exceed this TDI. This discussion of the WHO guideline value opens many questions for operators of water treatment plants. Because the guideline value is really only valid for microcystin-LR, in situations where it is not the most dominant congener or not even present, the evaluation of quantitative measurements with respect to the guideline may be problematic. This is true for HPLC analysis as well as for the mouse bioassay and the protein phosphatase inhibition assay (*11*). Results should thus always be reported with these points in mind and should, if possible, be reported for microcystin-LR concentration equivalents or toxicity equivalents. The next question that obviously arises is which water treatment procedures are adequate to reduce cyanotoxin levels to at least below the WHO guideline value of 1.0 µg/L?

Assessment of water treatment procedures has shown that most methods would result in a reduction of cyanobacterial toxins concentrations to below acutely toxic levels as well as below the new WHO guideline value of 1 µg/L drinking water. A completely different situation may arise, however, during a bloom and when water treatment procedures such as chlorination and/or activated carbon are not used together. Even when using ozonation, the specific situation during a bloom has to be observed. Parameters such as organic load of the water have to be determined and toxin levels during the treatment steps have to be monitored. Until issues such as ozonolysis byproducts have been resolved, even a very efficient method such as ozonation has to be treated with caution. These byproducts, which may especially be formed when an insufficient ozone dose has been used, have been detected in several studies, but neither their structure nor their toxicity has been determined. Binding to and inhibition of protein phosphatases is considered a key mechanism by which microcystins and nodularins exhibit their toxicity. It is therefore critical to know which structural modification to the toxin molecule changes the affinity to and inhibition of the phosphatase. So far, neither the Adda residue alone (*74,134*) nor linear nodularin- or microcystin-precursor peptides (*135*) bind protein phosphatase or bind with high IC_{50} values of 0.5–1.0 mM and show no toxicity in the mouse bioassay. However, it has also been shown that substitution of the Adda side chain with an L-Cys residue still leads to interaction of the toxin with the hydrophobic groove of the catalytic subunit of the phosphatase (*135*). The mechanism of tumor promotion by microcystins and nodularins as well as the quantitative relationships have not been satisfactorily elucidated. It is therefore not clear if the inhibition of protein phosphatase constitutes the only or major pathways for toxicity or tumor promotion. Our work has shown that in hepatocytes, microcystin binds to proteins other than the phosphatases (*136,137*). A chronic exposure to cyanobacterial toxins and/or to the ozonolysis byproducts should therefore be avoided. The situation for the saxitoxins, anatoxin-a, anatoxin-a(s), and cylindrospermopsin is even less clear. A broader scientific background on which risk assessment and management steps are based should be developed. This can lead to sound process-based risk assessment and to the development of effective procedures for water treatment strategies aimed at specific situations.

References and Notes

1. Francis G. Poisonous Australian lake. Nature 18:11–12 (1878).
2. Yu S-Z. Drinking water and primary liver cancer. In: Primary Liver Cancer (Tang ZY, Wu MC, Xia SS, eds). New York:China Academic Publishers/Springer, 1989;30–37.
3. Harada K, Oshikata M, Uchida H, Suzuki M, Kondo F, Sato K, Ueno Y, Yu SZ, Chen G, Chen GC. Detection and identification of microcystins in the drinking water of Haimen City, China. Nat Toxins 4:277–283 (1996).
4. Ueno Y, Nagata S, Tsutsumi T, Hasegawa A, Watanabe MF, Park H-D, Chen G-C, Chen G, Yu S-Z. Detection of microcystins, a blue-green algal hepatotoxin, in drinking water sampled in Haimen and Fusui, endemic areas of primary liver cancer in China, by highly sensitive immunoassay. Carcinogenesis 17:1317–1321 (1996).
5. Jochimsen EM, Carmichael WW, An JS, Cardo DM, Cookson ST, Holmes CE, Antunes MB, de Melo Filho DA, Lyra TM, Barreto VS, et al. Liver failure and death after exposure to microcystins at a hemodialysis center in Brazil . N Engl J Med 338:873–878 (1998). [Published erratum appears in N Engl J Med 339(2):139 (1998)].
6. Pouria S, de Andrade A, Barbosa J, Cavalcanti R, Barreto V, Ward C, Preiser W, Poon G, Neild G, Codd G. Fatal microcystin intoxication in haemodialysis unit in Caruaru, Brazil. Lancet 352:21–26 (1998).
7. Yoo SR, Carmichael WW, Hoehn RC, Hrudey SE. Cyanobacterial (Blue-Green Algal) Toxins: A Resource Guide:AWWA Research Foundation and American Water Works Association, 1995.
8. Whitton B. Diversity, ecology and taxonomy of the cyanobacteria. In: Photosynthetic Prokaryotes (Mann H, Carr N, eds). New York:Plenum Press, 1992;1–51.
9. Castenholz RW, Waterbury JB. Oxygenic photosynthetic bacteria. Group 1. Cyanobacteria. In: Bergey's Manual of Systematic Bacteriology, Vol 3 (Stanley JT, Bryant MP, Pfennig N, Holt JG, eds). Baltimore, 1989;1710–1806.
10. Skulberg OM, Carmichael WW, Codd GA, Skulberg R. Taxonomy of toxic cyanophyceae (Cyanobacteria). In: Algal Toxins in Seafood and Drinking Water (Falconer IR, ed). London:Academic Press, 1993;145–164.
11. Chorus I, Bartram J. Toxic Cyanobacteria in Water. A Guide to Their Public Health Consequences, Monitoring and Management. Geneva:World Health Organization, 1999.
12. Paerl H, Millie D. Physiological ecology of toxic aquatic cyanobacteria. Phycologia 35:160–167 (1996).
13. Carmichael WW, Gorham PR. Factors influencing the toxicity and animal susceptibility of *Anabaena flos-aquae* (Cyanophyta) blooms. J Phycol 13:97–101 (1977).
14. Rapala J, Sivonen K, Lyra C, Niemelä SI. Variation of microcystins, cyanobacterial hepatotoxins, in *Anabaena* spp. as a function of growth stimuli. Appl Environ Microbiol 63:2206–12 (1997).
15. Lehtimäki J, Sivonen K, Luukainen R, Niemelä SI. The effects of incubation time, temperature, light, salinity, and phosphorus on growth and hepatotoxin production by *Nodularia* strains. Arch Hydrobiol 130:269–282 (1994).
16. Sivonen K. Effects of light, temperature, nitrate, orthophosphate, and bacteria on growth of and hepatotoxin production by *Oscillatoria agardhii* strains. Appl Environ Microbiol 56:2658–66 (1990).
17. Botes DP, Kruger H, Viljoen CC. Isolation and characterization of four toxins from the blue-green alga *Microcystis aeruginosa*. Toxicon 20:945–954 (1982).
18. Namikoshi M, Sivonen K, Evans WR, Carmichael WW, Sun F, Rouhiainen L, Luukkainen R, Rinehart KL. Two new L-serine variants of microcystins-LR and -RR from *Anabaena* sp. strains 202 A1 and 202 A2. Toxicon 30:1457–1464 (1992).
19. Meriluoto JAO, Sandström A, Eriksson JE, Remaud G, Craig AG, Chattopadhyaya J. Structure and toxicity of a peptide hepatotoxin from the cyanobacterium *Oscillatoria agardhii*. Toxicon 27:1021–1034 (1989).
20. Namikoshi M, Rinehart KL, Sakai R, Sivonen K, Carmichael WW. Structures of three new cyclic heptapeptide hepatotoxins procuced by the cyanobacterium (blue-green alga) *Nostoc* sp. strain 152. J Org Chem 55:6135–6139 (1990).
21. Carmichael WW, Eschedor JT, Patterson GML, Moore RE. Toxicity and partial structure of a hepatotoxic peptide produced by the cyanobacterium *Nodularia spumigena* Mertens emend. L 575 from New Zealand. Appl Environ Microbiol 54:2257–2263 (1988).
22. Dittmann E, Neilan B, Erhard M, von Döhren H, Börner T. Insertional mutagenesis of a peptide synthetase gene that is responsible for hepatotoxin production in the cyanobacterium *Microcystis aeruginosa* PCC 7806. Mol Microbiol 26:779–787 (1997).
23. Meissner K, Dittmann E, Börner T. Toxic and non-toxic strains of the cyanobacterium *Microcystis aeruginosa* contain sequences homologous to peptide synthetase genes. FEMS Microbiol Lett 135:295–303 (1996).
24. Neilan BA, Jacobs D, Goodman AE. Genetic diversity and phylogeny of toxic cyanobacteria determined by DNA polymorphisms within the phycocyanin locus. Appl Environ Microbiol 61:3875–3883 (1995).
25. Neilan BA, Jacobs D, Del Dot T, Blackall LL, Hawkins PR, Cox PT, Goodman AE. rRNA sequences and evolutionary relationships among toxic and nontoxic cyanobacteria of the genus. Int J Syst Bacteriol 47:693–697 (1997).
26. Rudi K, Skulberg OM, Larsen F, Jakobsen KS. Quantification of toxic cyanobacteria in water by use of competitive PCR followed by sequence-specific labeling of oligonucleotide probes. Appl Environ Microbiol 64:2639–2643 (1998).
27. Botes DP, Viljoen CC, Kruger H, Wessels PL, Williams DH. Configuration assignments of the amino acid residues and the presence *N*-methyldehydroalanine in toxins from the blue-green alga, *Microcystis aeruginosa*. Toxicon 20:1037–1042 (1982).
28. Runnegar MTC, Falconer IR, Silver J. Deformation of isolated rat hepatocytes by a peptide hepatotoxin from the blue-green alga *Microcystis aeruginosa*. Naunyn-Schmiedeberg's Arch Pharmacol 317:268–272 (1981).
29. Runnegar MTC, Falconer IR. The *in vivo* and *in vitro* biological effects of the peptide hepatotoxin from the blue-green alga *Microcystis aeruginosa*. S Afr J Sci 78:363–366 (1982).
30. Eriksson JE, Grönberg L, Nygård S, Slotte JP, Meriluoto JAO. Hepatocellular uptake of ^{3}H-dihydromicrocystin-LR, a cyclic peptide toxin. Biochim Biophys Acta 1025:60–66 (1990).
31. Botes DP, Wessels PL, Kruger H, Runnegar MTC, Santikarn S, Smith RJ, Barna JCJ, Williams DH. Structural studies on cyanoginosins-LR, -YR, -YA, and -YM, peptide toxins from *Microcystis aeruginosa*. J Chem Soc, Perkin Trans 1:2747–2748 (1985).
32. Rinehart KL, Namikoshi M, Choi BW. Stucture and biosynthesis of toxins from blue-green algae (cyanobacteria). J Appl Phycol 6:159–176 (1994).
33. Sivonen K. Cyanobacterial toxins and toxin production. Phycologia 35:12–24 (1996).
34. Carmichael WW, Beasley V, Bunner DL, Eloff JN, Falconer I, Gorham P, Harada K-I, Yu M-J, Krishnamurthy T, Moore RE, et al. Naming of cyclic heptapeptide toxins of cyanobacteria (blue-green algae) [Letter to the Editor]. Toxicon 26:971–973 (1988).
35. Hawkins PR, Runnegar MTC, Jackson ARB, Falconer IR. Severe

hepatotoxicity caused by the tropical cyanobacterium (blue-green alga) – (Woloszynska) Seenaya and Subba Raju isolated from a domestic water supply reservoir. Appl Environ Microbiol 50:1292–1295 (1985).
36. Carmichael WW, Briggs DF, Gorham PR. Toxicology and pharmacological action of *Anabaena flos-aquae* toxin. Science 187:542–544 (1975).
37. Park H-D, Watanabe MF, Harada K-I, Nagai H, Suzuki M, Watanabe M, Hayashi H. Hepatotoxin (microcystin) and neurotoxin (anatoxin-a) contained in natural blooms and strains of cyanobacteria from Japanese freshwaters. Nat Toxins 1:353–360 (1993).
38. Matsunaga S, Moore R, Niemczura W, Carmichael W. Anatoxin-a(s), a potent anticholinesterase from *Anabaena flos-aquae*. J Am Chem Soc 111:8021–8023 (1989).
39. Carmichael WW, Mahmood NA, Hyde EG. Natural toxins from cyanobacteria. In: Marine Toxins, Origin, Structure, and Molecular Pharmacology. Vol ACS Symposium Series 418 (Hall S, Strichartz G, eds). Washington, DC:American Chemical Society, 1990;87–106.
40. Negri AP, Jones GJ. Bioaccumulation of paralytic shellfish poisoning (PSP) toxins from the cyanobacterium *Anabaena circinalis* by the freshwater mussel *Alathyria condola*. Toxicon 33:667–678 (1995).
41. Mahmood NA, Carmichael WW. Paralytic shellfish poisons produced by the freshwater cyanobacterium *Aphanizomenon flos-aquae* NH-5. Toxicon 24:175–186 (1986).
42. Humpage A, Rositano J, Bretag A, Brown R, Baker P, Nicholson B, Steffensen D. Paralytic shellfish poisons from Australian cyanobacterial blooms. Aust J Mar Freshwat Res 45:761–771 (1994).
43. Andersen RJ, Luu HA, Chen DZX, Holmes CFB, Kent ML, Le Blanc M, Taylor FJR, Williams DE. Chemical and biological evidence links microcystins to salmon 'netpen liver disease'. Toxicon 31:1315–1323 (1993).
44. Rodger HD, Turnbull T, Edwards C, Codd GA. Cyanobacterial (blue-green-algal) bloom associated pathology in brown trout, *Salmo trutta* L., in Loch Leven, Scotland. J Fish Dis 17:177–181 (1994).
45. Toranzo AE, Nieto F, Barja JL. Mortality associated with cyanobacterial bloom in farmed rainbow trout in Galicia (Northwestern, Spain). Bull Eur Assoc Fish Pathol 10:106–107 (1990).
46. Penaloza R, Rojas M, Vila I, Zambrano F. Toxicity of a soluble peptide from *Microcystis* sp. to zooplankton and fish. Freshwater Biol 24:233–240 (1990).
47. Devidze M. Harmful algal events in Georgian waters. In: Harmful Algae (Reguera B, Blanco J, Fernández L, Wyatt T, eds). Vigo, Spain:Xunta de Galicia, Paris, France: Intergovernmental Oceanographic Commission of UNESCO, 1998;91.
48. Falconer IR, Buckley T, Runnegar MT. Biological half-life, organ distribution and excretion of ^{125}I-labelled toxic peptide from the blue-green alga *Microcystis aeruginosa*. Aust J Biol Sci 39:17–21 (1986).
49. Brooks WP, Codd GA. Distribution of *Microcystis aeruginosa* peptide toxin and interactions with hepatic microsomes in mice. Pharmacol Toxicol 60:187–191 (1987).
50. Runnegar MTC, Falconer IR, Buckley T, Jackson ARB. Lethal potency and tissue distribution of 125I-labelled toxic peptides from the blue-green alga *Microcystis aeruginosa*. Toxicon 24:506–509 (1986).
51. Robinson NA, Miura GA, Matson CF, Dinterman RE, Pace JG. Characterization of chemically tritiated-microcystin-LR and its distribution in mice. Toxicon 27:1035–1042 (1989).
52. Lin J-R, Chu FS. Kinetics of distribution of microcystin LR in serum and liver cytosol of mice: an immunochemical analysis. J Agric Food Chem 42:1035–1040 (1994).
53. Stotts RR, Twardock AR, Koritz GD, Haschek WM, Manuel RK, Hollis WB, Beasley VR. Toxicokinetics of tritiated dihydromicrocystin-LR in swine. Toxicon 35:455–465 (1997).
54. Stotts RR, Twardock AR, Haschek WM, Choi BW, Rinehart KL, Beasley VR. Distribution of tritiated dihydromicrocystin in swine. Toxicon 35:937–953 (1997).
55. Falconer IR, Smith JV, Jackson ARB, Jones A, Runnegar MTC. Oral toxicity of a bloom of the cyanobacterium *Microcystis aeruginosa* administered to mice over periods up to 1 year. J Toxicol Environ Health 24:291–305 (1988).
56. Falconer I, Burch M, Steffensen D, Choice M, Coverdale O. Toxicity of the blue-green alga (cyanobacterium) *Microcystis aeruginosa* in drinking water to growing pigs, as an animal model for human injury and risk assessment. J Environ Toxicol Water Quality 9:131–139 (1994).
57. Hooser SB, Beasley VR, Lovell RA, Carmichael WW, Haschek WM. Toxicity of microcystin-LR, a cyclic heptapeptide from *Microcystis aeruginosa*, to rats and mice. Vet Pathol 26:246–252 (1989).
58. Runnegar MT, Maddatu T, Deleve LD, Berndt N, Govindarajan S. Differential toxicity of the protein phosphatase inhibitors microcystin and calyculin a. J Pharmacol Exp Ther 273:545–553 (1995).
59. Wickstrom M, Haschek W, Henningsen G, Miller LA, Wyman J, Beasley V. Sequential ultrastructural and biochemical changes induced by microcystin-LR in isolated perfused rat livers. Nat Toxins 4:195–205 (1996).
60. Ito E, Kondo F, Harada KI. Hepatic necrosis in aged mice by oral administration of microcystin-LR. Toxicon 35:231–239 (1997).
61. Yoshida T, Makita Y, Tsutsumi T, Yoshida F, Sekijima M, Tamura S-i, Ueno Y. Acute oral toxicity of microcystin-LR, a cyanobacterial hepatotoxin, in mice. Nat Toxins 5:91–95 (1997).
62. Matsushima R, Yoshizawa S, Watanabe MF, Harada K, Furusawa M, Carmichael WW, Fujiki H. *In vitro* and *in vivo* effects of protein phosphatase inhibitors, microcystins and nodularin, on mouse skin and fibroblasts. Biochem Biophys Res Commun 171:867–74 (1990).
63. Yoshizawa S, Matsushima R, Watanabe MF, Harada K-I, Ichihara A, Carmichael WW, Fujiki H. Inhibition of protein phosphatases by microcystin and nodularin associated with hepatotoxicity. J Cancer Res Clin Oncol 116:609–614 (1990).
64. Toivola DM, Eriksson JE, Brautigan DL. Identification of protein phosphatase 2A as the primary target for microcystin-LR in rat liver homogenates. FEBS Lett 344:175–80 (1994).
65. Runnegar M, Berndt N, Kong SM, Lee EY, Zhang L. *In vivo* and *in vitro* binding of microcystin to protein phosphatases 1 and 2A. Biochem Biophys Res Commun 216:162–169 (1995).
66. MacKintosh RW, Dalby KN, Campbell DG, Cohen PT, Cohen P, MacKintosh C. The cyanobacterial toxin microcystin binds covalently to cysteine-273 on protein phosphatase 1. FEBS Lett 371:236–240 (1995).
67. Eriksson JE, Paatero GIL, Meriluoto JAO, Codd GA, Kass GEN, Nicotera P, Orrenius S. Rapid microfilament reorganization induced in isolated rat hepatocytes by microcystin-LR, a cyclic peptide toxin. Exp Cell Res 185:86–100 (1989).
68. Eriksson JE, Toivola D, Meriluoto JAO, Karaki H, Han Y-G, Hartshorne D. Hepatocyte deformation induced by cyanobacterial toxins reflects inhibition of protein phosphatases. Biochem Biophys Res Commun 173:1347–1353 (1990).
69. Eriksson JE, Golman RD. Protein phosphatase inhibitors alter cytoskeletal structure and cellular morphology. Adv Prot Phosphatases 7:335–357 (1993).
70. Runnegar MTC, Falconer IR. Effect of toxin from the cyanobacterium *Microcystis aeruginosa* on ultrastructural morphology and actin polymerization in isolated hepatocytes. Toxicon 24:109–115 (1986).
71. Runnegar MTC, Andrews J, Gerdes RG, Falconer IR. Injury to hepatocytes induced by a peptide toxin from the cyanobacterium *Microcystis aeruginosa*. Toxicon 25:1235–1239 (1987).
72. Craig M, Luu HA, McCready TL, Williams D, Andersen RJ, Holmes C. Molecular mechanisms underlying the interaction of motuporin and microcystins with type-1 and type-2A protein phosphatases. Biochem Cell Biol - Biochim Biol Cell 74:569–578 (1996).
73. Rudolph-Böhner S, Mierke DF, Moroder L. Molecular structure of the cyanobacterial tumor-promoting microcystins. FEBS Lett 349:319–323 (1994).
74. Namikoshi N, Rinehart K, Dahlem A, Beasley V, Carmichael W. Total synthesis of Adda, the unique C_{20} amino acid of cyanobacterial hepatotoxins. Tetrahedron Lett 30:4349–4352 (1989).
75. Fujiki H, Suganuma M. Unique features of the okadaic acid activity class of tumor promoters. J Cancer Res Clin Oncol 125:150–155 (1999).
76. Falconer IR. Tumor promotion and liver injury caused by oral consumption of cyanobacteria. Environ Toxicol Water Qual 6:177–184 (1991).
77. Fujiki H, Suganuma M, Yoshizawa S, Kanazawa H, Sugimura T, Manam S, Kahn SM, Jiang W, Hoshina S, Weinstein IB. Codon 61 mutations in the c-Harvey-*ras* gene in mouse skin tumors induced by 7,12-dimethylbenz(a)anthracene plus okadaic acid class tumor promoters. Mol Carcinog 2:184–187 (1989).
78. Fujiki H. Is the inhibition of protein phosphatase 1 and 2A activities a general mechanism of tumor promotion in human cancer development? Mol Carcinog 5:91–94 (1992).
79. Nishiwaki-Matsushima R, Ohta T, Nishiwaki S, Suganuma M, Kohyama K, Ishikawa T, Carmichael WW, Fujiki H. Liver tumor promotion by the cyanobacterial cyclic peptide toxin microcystin-LR. J Cancer Res Clin Oncol 118:420–424 (1992).
80. Ohta T, Sueoka E, Iida N, Komori A, Suganuma M, Nishiwaki R, Tatematsu M, Kim SJ, Carmichael WW, Fujiki H. Nodularin, a potent inhibitor of protein phosphatases 1 and 2A, is a new environmental carcinogen in male F344 rat liver. Cancer Res 54:6402–6406 (1994).
81. Ito E, Kondo F, Terao K, Harada K-I. Neoplastic nodular formation in mouse liver induced by repeated intraperitoneal injections of microcystin-LR. Toxicon 35:1453–1457 (1997).
82. Sueoka E, Sueoka N, Okabe S, Kozu T, Komori A, Ohta T, Suganuma M, Kim SJ, Lim IK, Fujiki H. Expression of the tumor necrosis factor alpha gene and early response genes by nodularin, a liver tumor promoter, in primary cultured rat hepatocytes. J Cancer Res Clin Oncol 123:413–419 (1997).
83. Suzuki H, Watanabe M, Wu Y, Sugita T, Kita K, Sato T, Wang X-L, Tanzawa H, Sekiya S, Suzuki N. Mutagenicity of microcystin-LR in human RSa cells. Int J Mol Med 2:109–112 (1998).
84. Rao PVL, Bhattacharya R. The cyanobacterial toxin microcystin-LR induced DNA damage in mouse liver *in vivo*. Toxicology 114:29–36 (1996).
85. Rao P, Bhattacharya R, Parida MM, Jana AM, Bhaskar A. Freshwater cyanobacterium *Microcystis aeruginosa* (UTEX 2385) induced DNA damage *in vivo* and *in vitro*. Environ Toxicol Pharmacol 5:1–6 (1998).
86. Tisdale ES. Epidemic of intestinal disorders in Charleston, W. VA., occurring simultaneously with unprecedented water supply conditions. Am J Public Health 21:198–200 (1931).
87. Zilberg B. Gastroenteritis in Salisbury European children-a five-year study. Cent Afr J Med 12:164–168 (1966).
88. Falconer IR, Beresford AM, Runnegar MTC. Evidence of liver damage by toxin from a bloom of the blue-green alga, *Microcystis aeruginosa*. Med J Aust 1:511–514 (1983).
89. Teixera M, Costa M, Carvalho V, Pereira M, Hage E. Gastroenteritis epidemic in the area of the Itaparica Dam, Bahia, Brazil. Bull Pan Am Health Org 27:244–253 (1993).
90. James H, Fawell J. Detection and removal of cyanobacterial toxins from freshwaters FR 0211. Murlow, Buckinghamshire, UK:Foundation for Water Research, 1991.
91. Rositano J, Nicholson B. Water Treatment Techniques for the Removal of Cyanobacterial Toxins from Water 2/94. Salisbury, S.A., Australia:Australian Centre for Water Quality Research, 1994.
92. Mouchet P, Bonnélye V. Solving algae problems: French expertise and world-wide applications. J Water SRT - Aqua 47:125–141 (1998).
93. Drikas M, Hrudey S. Control and removal of toxins: Summary of discussions. In: Toxic Cyanobacteria. Current Status of Research and Management, March 22–26, 1994, Adelaide, Australia. (Steffensen D, Nicholson B, eds). Denver, CO:American Water Works Association Research Foundation, 1994.
94. Hoffmann J. Removal of *Microcystis* toxins in water purification processes. Water SA 2:58–60 (1976).
95. Keijola AM, Himberg K, Esala AL, Sivonen K, Hiisvirta L. Removal of cyanobacterial toxins in water treatment processes: laboratory and pilot-scale experiment. Toxicity Assess 3:643–656 (1988).
96. Himberg K, Keijola A-M, Hiisvirta L, Pyysalo H, Sivonen K. The effect of water treatment processes on the removal of hepatotoxins from *Microcystis* and *Oscillatoria* cyanobacteria: a laboratory study. Water Res 23:979–984 (1989).
97. Lahti K, Hiisvirta L. Removal of cyanobacterial toxins in water treatment processes: review of studies conducted in Finland. Water Supply 7:149–154 (1989).
98. Lepistö L, Lakti K, Niemi J. Removal of cyanobacteria and other phytoplankton in four Finnish waterworks. Algolog Studies 75:167–181 (1994).
99. Falconer IR, Runnegar MTC, Huynh VL. Effectiveness of activated carbon in the removal of algal toxin from potable water supplies: a pilot plant investigation. In: Technical Papers, Tenth Federal Convention of the Australian Water and Wastewater Association, Sydney, Australia. 1983;1–8.
100. Falconer I, Runnegar M, Buckley T, Huyn V, Bradshaw P. Using activated carbon to remove toxicity from drinking water containing cyanobacterial blooms. J Am Water Works Association 81:102–105 (1989).
101. Hart J, Stott P. Microcystin-LR Removal from Water FR 0367. Murlow, Buckinghamshire, UK:Foundation for Water Research, 1993.
102. Donati C, Drikas M, Hayes R, Newcombe G. Microcystin-LR adsorption by powdered activated carbon. Water Res 28:1735–1742 (1994).
103. Lambert TW, Holmes CFB, Hrudey SE. Adsorption of microcystin-LR by activated carbon and removal in full scale water treatment. Water Res 30:1411–1422 (1996).
104. Zurich Water Works, pesonal communication.
105. Steffensen DA, Nicholson BC. Toxic cyanobacteria current status of reseach and management. In: Toxic Cyanobacteria Current Status of Reseach and Management, March 22–26,

1994, Adelaide, Australia. (Steffensen D, Nicholson B, eds). Denver, CO: American Water Works Association Research Foundation, 1994.
106. Höger S, Dietrich D, Hitzfeld B. Effect of ozonation in drinking water treatment on the removal of cyanobacterial toxins [Abstract]. Toxicol Sci 48:33 (1999).
107. Carlile P. Further studies to investigate microcystin-LR and anatoxin-A removal from water FR 0458: Murlow, Buckinghamshire, UK:Foundation for Water Research, 1994.
108. Nicholson B, Rositano J, Humpage A, Burch M. Removal of algal toxins in water treatment processes. In: 15th AWWA Federal Convention, Gold Coast, Queensland, Australia. Sydney, Australia:Australian Water and Wastewater Association. 1993;327–331.
109. Nicholson BC, Rositano J, Burch MD. Destruction of cyanobacterial peptide hepatotoxins by chlorine and chloramine. Water Res 28:1297–1303 (1994).
110. Nicholson B, Rositano J. Chemical methods for the destruction of cyanobacterial toxins. In: Workshop on Cyanobacteria (Blue-Green Algae) and their Toxins, Brisbane, Australia, 1997. Sydney, Australia:Australian Water and Wastewater Association, 1997.
111. Rositano J, Bond P, Nicholson B. By-products of the destruction of cyanobacterial peptide hepatotoxins using chlorine. In: 16th AWWA Federal Convention, Darling Harbour, Sydney, Australia. Sydney, Australia:Australian Water and Wastewater Association, 1995;937–942.
112. Tsuji K, Naito S, Kondo F, Ishikawa N, Watanabe MF, Suzuki M, Harada K-I. Stability of microcystins from cyanobacteria: effect of light on decomposition and isomerization. Environ Sci Technol 28:173–177 (1994).
113. Tsuji K, Watanuki T, Kondo F, Watanabe M, Suzuki S, Nakazawa H, Suzuki M, Uchida H, Harada K-I. Stability of microcystins from cyanobacteria. II: Effect of UV light on decomposition and isomerization. Toxicon 33:1619–1631 (1995).
114. Shephard G, Stockenström S, de Villiers D, Engelbrecht W, Sydenham E, Wessels G. Photocatalytic degradation of cyanobacterial microcystin toxins in water. Toxicon 36:1895–1901 (1998).
115. Chow C, Panglisch S, Mole J, Drikas M, Burch M, Gimbel R. A study of membrane filtration for the removal of cyanobacterial cells. J Water SRT - Aqua 46:324–334 (1997).
116. Muntisov M, Trimboli P. Removal of algal toxins using membrane technology [Technical note]. Water 23:34 (1996).
117. Langlais B, Reckhow DA, Brink DR. Ozone in Water Treatment. Application and Engineering. Denver, CO/Chelsea, MI:American Water Works Association Research Foundation/Lewis Publishers, 1991.
118. Masten S, Davies S. The use of ozonation to degrade organic contaminants in wastewaters. Environ Sci Technol 28:180A–185A (1994).
119. James H, Smith C, Sutton A. Levels of anatoxin-A and microcystin-LR in raw and treated waters FR 0460. Murlow, Buckinghamshire, UK:Foundation for Water Research, 1994.
120. Rositano J, Nicholson B, Pieronne P. Destruction of cyanobacterial toxins by ozone. Ozone Sci Eng 20:223–238 (1998).
121. Höger SJ, Hitzfeld BC, Dietrich DR. Unpublished data (1999).
122. Bernazeau F. Can microcystins enter drinking water distribution systems? In: Toxic Cyanobacteria. Current Status of Research Management., Adelaide, Australia. (Steffensen D, Nicholson B, eds). Denver, CO: American Water Works Association Research Foundation, 1994;115–118.
123. Harada K. Chemistry and detection of microcystins. In: Toxic Microcystis (Watanabe M, Harada K, Carmichael W, Fujiki H, eds). Boca Raton, FL:CRC Press, 1996;103–148.
124. Lawrence J, Tosine H, Onuska F, Comba M. The ozonation of natural waters: product identification. Ozone Sci Eng 2:55–64 (1980).
125. Rinehart KL, Harada K-I, Namikoshi M, Chen C, Harvis CA, Munro MHG, Blunt JW, Mulligan PE, Beasley BR, Dahlem AM, et al. Nodularin, microcystin, and the configuration of Adda. J Am Chem Soc 110:8557–8558 (1988).
126. Harada K-I, Murata H, Qiang Z, Suzuki M, Kondo F. Mass spectrometric screening method for microcystins in cyanobacteria. Toxicon 34:701–710 (1996).
127. Falconer I. Potential impact on human health of toxic cyanobacteria. Phycologia 35:6–11 (1996).
128. Falconer I. An overview of problems caused by toxic blue-green algae (cyanobacteria) in drinking water. Environ Toxicol 14:5–12 (1999).
129. Pilotto L, Douglas R, Burch M, Cameron S, Beers M, Rouch G, Robinson P, Kirk M, Cowie C, Hardiman S, et al. Health effects of exposure to cyanobacteria (blue-green algae) during recreational water-related activities. Aust N Z J Public Health 21:562–566 (1997).
130. Gilroy D, Chu F. Deriving a safe level for microcystin toxin in blue-green algae dietary supplements [Abstract]. Toxicol Sci 42:227 (1998).
131. WHO. Guidelines for Drinking-Water Quality. Addendum to Vol 2. Geneva:World Health Organization, 1998.
132. Fawell J, James C, James H. Toxins from blue-green algae: Toxicological assessment of microcystin-LR and a method for its determination in water FR0358/2/DoE 3. Murlow, Buckinghamshire, UK:Foundation for Water Research, 1994.
133. Kuiper-Goodmann T. Risk assessment of microcystins in Canada. WaBoLu-Hefte 4/97:9–12 (1997).
134. Choi BW, Namikoshi M, Sun F, Rinehart KL, Carmichael WW, Kaup AM, Evans WR, Beasley VR. Isolation of linear peptides related to the hepatotoxins nodularin and microcystins. Tetrahedron Lett 34:7881–7884 (1993).
135. Taylor C, Quinn RJ, Suganuma M, Fujiki H. Inhibition of protein phosphatase 2A by cyclic peptides modelled on the microcystin ring. Bioorg Med Chem Lett 6:2113–2116 (1996).
136. Hitzfeld BC, Fischer WF, Eriksson JE, Mikhailov A, Dietrich DR. Immunochemical detection of microcystin-LR in tissues and cells of rainbow trout [Abstract]. Toxicol Sci 48:33 (1999).
137. Hitzfeld B, Fischer W, Eriksson J, Mikhailov A, Tencalla F, Dietrich DR. Toxins of cyanobacteria in fish: immunohistochemical and immunocytochemical localization in livers and hepatocytes of rainbow trout [Abstract]. Naunyn-Schmiedeberg´s Arch Pharmacol 359:R159 (1999).
138. Botes DP, Tuinman AA, Wessels PL, Viljoen CC, Kruger H, Williams DH, Santikarn S, Smith RJ, Hammond SJ. The structure of cyanoginosin-LA, a cyclic heptapeptide toxin from the cyanobacterium *Microcystis aeruginosa*. J Chem Soc Perkin Trans 1:2311–2318 (1984).
139. Sivonen K, Namikoshi M, Evans WR, Carmichael WW, Sun F, Rouhiainen L, Luukkainen R, Rinehart KL. Isolation and characterization of a variety of microcystins from seven strains of the cyanobacterial genus *Anabaena*. Appl Environ Microbiol 58:2495–2500 (1992).
140. Painuly P, Perez R, Fukai T, Shimizu Y. The structure of a cyclic peptide toxin, cyanogenosin-RR from *Microcystis aeruginosa*. Tetrahedron Lett 29:11–14 (1988).
141. Kusumi T, Ooi T, Watanabe M, Takahashi H, Kakisawa H. Cyanoviridin RR, a toxin from the cyanobacterium (blue-green alga) *Microcystis viridis*. Tetrahedron Lett 28:4695–4698 (1987).
142. Krishnamurthy T, Szafraniec L, Hunt DF, Shabanowitz J, Yates III JR, Hauer CR, Carmichael WW, Skulberg O, Codd GA, Missler S. Structural characterization of toxic cyclic peptides from blue-green algae by tandem mass spectrometry. Proc Natl Acad Sci USA 86:770–774 (1989).
143. Harada K-I, Matsuura K, Suzuki M, Watanabe MF, Oishi S, Dahlem AM, Beasley VR, Carmichael WW. Isolation and characterization of the minor components associated with microcystins LR and RR in the cyanobacterium (blue-green algae). Toxicon 28:55–64 (1990).
144. Luukkainen R, Sivonen K, Namikoshi M, Fardig M, Rinehart KL, Niemela SI. Isolation and identification of eight microcystins from thirteen *Oscillatoria agardhii* strains and structure of a new microcystin. Appl Environ Microbiol 59:2204–2209 (1993).
145. Sivonen K, Kononen K, Carmichael WW, Dahlem AM, Rinehart KL, Kiviranta J, Niemelä SI. Occurrence of the hepatotoxic cyanobacterium *Nodularia spumigena* in the Baltic Sea and structure of the toxin. Appl Environ Microbiol 55:1990–1995 (1989).
146. Namikoshi M, Choi BW, Sakai R, Sun F, Rinehart KL, Carmichael WW, Evans WR, Cruz P, Munro MHG, Blunt JW. New nodularins: a general method for structure assignment. J Org Chem 59:2349–2357 (1994).
147. James KJ, Sherlock IR, Stack MA. Anatoxin-a in Irish freshwater and cyanobacteria, determined using a new fluorimetric liquid chromatographic method. Toxicon 35:963–971 (1997).
148. Mahmood NA, Carmichael WW. Anatoxin-a(s), an anticholinesterase from the cyanobacterium *Anabaena flos-aquae* NRC-525-17. Toxicon 25:1221–1227 (1987).
149. Carmichael WW. Cyanobacteria secondary metabolites - the cyanotoxins [Review]. J Appl Bacteriol 72:445–459 (1992).
150. Ohtani I, Moore R, Runnegar M. Cylindrospermopsin, a potent hepatotoxin from the blue-green alga *Cylindrospermopsis raciborskii*. J Chem Soc 114:7941–7942 (1992).
151. Mez K, Hanselmann K, Naegeli H, Preisig H. Protein phosphatase-inhibiting activity in cyanobacteria from alpine lakes in Switzerland. Phycologia 36:133–139 (1996).
152. Bourke ATC, Hawes RB, Neilson A, Stallman ND. An outbreak of hepato-enteritis (the Palm Island Mystery Disease) possibly caused by algal intoxication. Toxicon 3:45–48 (1983).
153. Mahmood NA, Carmichael WW, Pfahler D. Anticholinesterase poisonings in dogs from a cyanobacterial (blue-green algae) bloom dominated by *Anabaena flos-aquae*. Am J Vet Res 49:500–503 (1988).
154. Turner P, Gammie A, Hollinrake K, Codd G. Pneumonia associated with contact with cyanobacteria. British Medical Journal 300:1440–1441 (1990).
155. Edwards C, Beattie K, Scrimgour C, Codd G. Identification of anatoxin-a in benthic cyanobacteria (blue-green algae) and in associated dog poisonings at Loch Insh, Scotland. Toxicon 30:1165–1175 (1992).
156. Carmichael WW, Yu M-J, He Z-R, He J-W, Yu J-L. Occurrence of the toxic cyanobacterium (blue-green alga) *Microcystis aeruginosa* in Central China. Arch Hydrobiol 114:21–30 (1988).