Human Health Effects from Harmful Algal Blooms: a Synthesis

Submitted by the HPAB to the International Joint Commission
November 22, 2013

Preface

Harmful algal blooms have increased in frequency and severity over the past several years in Lake Erie and other parts of the Great Lakes basin. Meanwhile, environmental health scientists have been describing the potential human health impacts associated with harmful algal blooms. The revised Great Lakes Water Quality Agreement puts more emphasis on preventing human health effects from the waters of the Great Lakes. As a result of all these factors, the International Joint Commission's Health Professionals Advisory Board (HPAB) wanted to synthesize the existing literature on the human health effects from harmful algal blooms. By summarizing the current state of science on this topic, other environmental health scientists or subsequent work by HPAB can further the understanding of this human health concern, raise awareness, and protect the public. The Commission provided resources for a contractor to conduct the literature review with the oversight of the Board. Several public health experts in harmful algal blooms, a subset of Health Board members, a Science Advisory Board member, and a Commission staff member contributed to the report. The report was approved unanimously by all the members of the Health Professionals Advisory Board.

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Section H, Acknowledgements provides contact information for the individuals that made this report possible.

Executive Summary

This report addresses the objective to assess the human health impacts associated with harmful algal blooms (HABs) especially those associated with blue-green algae or cyanobacteria blooms (cyanoHABs). This report was prepared by the International Joint Commission's Health Professionals Advisory Board. The objective of the report is to summarize the state of science on this topic to help other environmental health scientists or subsequent work by HPAB to further the understanding of this human health concern, raise awareness, and protect public health. The report was completed as part of an effort to Integrate Ecosystem & Human Health Surveillance Data and that work is part of a Commission project to Improve the Delivery of Great Lakes Scientific Information to managers and the public.

The North American Great Lakes located between the United States and Canada collectively provide drinking water for more than 24 million people. In addition, numerous smaller lakes provide recreational opportunities for inhabitants and visitors to the Great Lakes Basin. The severe eutrophication and degradation of these major ecosystems during the last century led to the establishment of one of the world's largest and longest-standing restoration initiatives, which has operated since 1987 under the binational Great Lakes Water Quality Agreement (GLWQA).

A major consequence of this eutrophication and degradation of the Great Lakes ecosystems is the production of massive concentrations of cyanobacteria termed blooms. In Lake Erie these blooms have been recognized since the 1970s. The "harmful" aspect of these blooms, from an environmental context, begins with a loss of water clarity that suppresses aquatic macrophytes, and negatively affects invertebrate and fish habitats. Bacterial decomposition of dying blooms may lead to oxygen depletion (hypoxia and anoxia), and subsequent fish kills. In addition, many cyanoHABs produce toxic secondary metabolites, the cyanotoxins, which can cause serious, acute intoxication in mammals (including humans) affecting the hepatopancreatic, digestive, endocrine, dermal, and nervous systems.

This report addresses the objective to assess the human health impacts associated with harmful algal blooms (HABs) especially those associated with blooms of cyanobacteria or blue-green algae (cyanoHABs). The relevant information for this report comes from the open literature, as well as unpublished reports, case histories and personal communications from agency personnel and the general public who have investigated and witnessed the consequences of cyanoHABs. Major findings from the literature review include:

1) Cyanobacteria are a normal part of all ecosystems. However due to their long evolutionary period they can respond to anthropogenic modifications of aquatic environments, including nutrient over-enrichment (eutrophication), water diversions, withdrawals, and salinization.
2) For the Great Lakes, nutrient enrichment is particularly important, with the bloom

problems of the 1960's and 1970's being primarily due to point-source pollution. Today nutrient loading is coming chiefly from agriculture (2/3) and 1/3 from urban and recreational sources. The current phosphorus increases are a major reason for blooms of the cyanotoxin-producing cyanobacterium *Microcystis*.

3) Each of the Great Lakes has documented cyanoHAB events but only Lake Erie has its indicator classification for HABs defined as fair to poor.
4) To date, cyanotoxins detected in the Great Lakes are the liver toxins microcystins and cylindrospermospin, and the neurotoxins anatoxin-a and saxitoxins (Paralytic Shellfish Toxins). Only in Lake Erie have all of these cyanotoxins been detected with microcystins being detected at moderate health risk levels. In Lake Ontario only microcystins have been detected.
5) Exposures to cyanotoxins have led to acute animal and human toxicity and acute lethal poisonings in animals and wildlife in many states and provinces of the Great Lakes area. Only for Lake Erie have a few cases of human illness and animal (dog) deaths been documented.
6) In the Great Lakes, cyanoHABs have caused economic losses to the fishing and recreation industries while increasing costs for the treatment of potable water supplies.

Contents

List of Acronyms

anatoxin-a (antx-a)
arginine (R)

ß-methylamino alanine (BMAA)

Centers for Disease Control (CDC)
cyanobacteria blooms (cyanoHABs)
Cyanobacteria-Like Bodies (CLB)
cylindrospermopsin (CYN)

gas chromatography/mass spectrometry (GC/MS)
Grand Lake St. Marys (GLSM)
Great Lakes Water Quality Agreement (GLWQA)

Harmful Algal Bloom-related Illness Surveillance System (HABISS)
harmful algal blooms (HABs)
health alert level (HAV)
Health Professionals Advisory Board (HPAB)
Hepatitis B virus (HBV)
hydroxysuccinimidyl carbamate (AQC)

International Code of Botanical Nomenclature (ICBN)
International Code of Nomenclature of Bacteria (ICNB)

leucine (L)
Lipopolysaccharides (LPS)
liquid chromatography/mass spectrometry (LC/MS)
Liquid chromatography-tandem mass spectrometry (LC/MS/MS)

lowest-observed-adverse-effect level (LOAEL)

maximum acceptable concentration (MAC)
microcystins (MCYSTs)
micrograms/liter (µg/L)

National Oceanic and Atmospheric Administration (NOAA)
National Outbreak Reporting System (NORS)
nitrogen (N)
nodularins (NODLNs)
no-observed-adverse-effect level (NOAEL)

paralytic shellfish toxins (PSTs)
parts per billion (ppb)
phosphorus (P)
provisional guidance value (P)GV
provisional maximum value or concentration (P)MAV / (P)MAC)

Risk management framework (RMF)

Standard (S)

tolerable daily intake (TDI)
total nitrogen (TN)
total phosphorus (TP)

United States Environmental Protection Agency (USEPA)
United States Geological Survey (USGS)

World Health Organization (WHO)

List of Tables and Figures

A. Cyanobacteria Harmful Algae Blooms (CyanoHABs)

1. Background

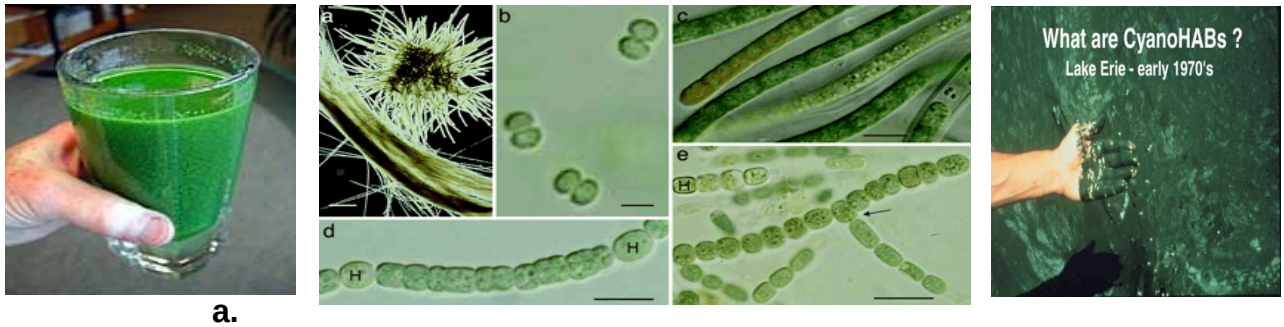
Cyanobacteria—also known as blue-green algae, blue-green bacteria, and cyanophytes—are normal components of our water resources (Figure 1). Their primary distinction from other algae is that they are prokaryotic. They were studied as microalgae by phycologists under the International Code of Botanical Nomenclature (ICBN) along with studies of the more numerous eukaryotic algae. Their prokaryotic nature and close relationship with eubacteria made work under provisions of the International Code of Nomenclature of Bacteria (ICNB) more appropriate. As such they are now referred to more commonly as cyanobacteria. The prokaryotic nature of cyanobacteria led to their being included in Bergey's Manual of Systematic Bacteriology (1989). However, the use of bacterial identification methods has its limitation for an organism that can be described fairly well using a light microscope. This has led to an attempt to combine the methods used by the phycologist with those used by the bacteriologist (Komárek and Anagnostidis 1998 and 2005).A useful taxonomic key for the toxigenic cyanobacteria can be found in Chapman (2010). A useful photo guide to aquatic cyanobacteria is available in Cronberg and Annadotter (2006).

Harmful algal blooms (HABs) are proliferations of microscopic algae that harm the environment by producing toxins that accumulate in shellfish or fish, or through the accumulation of biomass that in turn affects co-occurring organisms and alters food webs in negative ways. Impacts include human illness and mortality following direct consumption or indirect exposure to toxic shellfish or toxins in the environment; economic hardship for shoreline economies, many of which are highly dependent on tourism or harvest of local seafood; as well as dramatic fish, bird, and mammal mortalities. Equally important are the devastating impacts HABs may cause to ecosystems, leading to environmental damage that may reduce the ability of those systems to sustain species due to habitat degradation, increased susceptibility to disease, and long term alterations to community structure. In short, HABs lead to poisonous seafood, mortality of fish and other animals, economic impacts to shoreline communities, losses to aquaculture enterprises, and long-term ecosystem changes

Fresh, brackish and marine cyanobacteria (blue-green algae) all produce potent toxins. Cyanobacteria are the Earth's oldest oxygenic photoautotroph's and have had major impacts on shaping its biosphere. Their long evolutionary history (~3.5 billion years) has enabled them to adapt to geochemical and climatic changes, and more recently anthropogenic modifications of aquatic environments, including nutrient over-enrichment (eutrophication), water diversions, withdrawals, and salinization. Many cyanobacterial genera exhibit optimal growth rates and bloom potentials at relatively high water temperatures; hence global warming plays a key role in their expansion and persistence. Bloom-forming cyanobacterial taxa can be harmful from environmental, organismal, and human health perspectives by outcompeting beneficial phytoplankton, depleting oxygen upon bloom senescence, and producing a variety of toxic metabolites (e.g., cyanotoxins).

When dense algae populations develop, especially cyanobacteria, they turn water a green or greenish brown color referred to as a “bloom.” Blooms are simply high concentrations of algal cells that give the water a “pea soup” appearance. Dense blooms near the surface may resemble a layer of green paint (Figure1—Right Plate—Lake Erie, and Photograph on Title Page). Problem blooms occur in the summer and fall months and can be more frequent in times of drought, and as the number of algal cells in water increases, the chances for problems are also increased. The World Health Organization (WHO) defines their standard for problem blooms as populations of algal cells exceeding 100,000 cells/milliliter, or the equivalent of 24 million algal cells in an 8-ounce cup of water. Individual states may have different standards, and health agencies may test for specific toxins as well (Chorus and Bartram 1999).

Figure 1. Cyanobacteria Micrographs and CyanoHAB Photograph.
Left Plate. Drinking Glass With CyanoHAB
Middle Plate. Some Common Cyanobacteria.



a.
Colony of *Aphanizomenon* (left) and *Gloeotrichia* (top right),
b. *Gloeocapsa*,
c. *Planktothrix* (with sheath),
d-e. *Anabaena* H=heterocyst
Right Plate. Photograph of a Lake Erie bloom of *Aphanizomenon flos-aquae* (not a cyanotoxin producing species) from circa 1971.

The Harmful Algal Bloom and Hypoxia Amendments Act of 2004 mandates that the National Oceanic and Atmospheric Administration (NOAA) advance the scientific understanding and ability to detect, monitor, assess, and predict HABs and hypoxia events in coastal waters and the Great Lakes. Research and advances in knowledge have occurred regarding marine HABs. However, research on U.S. inland and fresh waters HABs has not been as extensive with the greatest federal efforts focused on the Great Lakes.

A major consequence of this eutrophication and degradation of the Great Lakes ecosystems is the production of massive concentrations of cyanobacteria termed blooms. In Lake Erie these blooms have been recognized since the 1970s. The “harmful” aspect of these blooms, from an environmental context, begins with a loss of water clarity that suppresses aquatic macrophytes, and negatively affects invertebrate and fish habitats. Bacterial decomposition of dying blooms may lead to oxygen

depletion (hypoxia and anoxia), and subsequent fish kills. In addition, many CyanoHABs produce toxic secondary metabolites, the cyanotoxins, which can cause serious, acute intoxication in mammals (including humans) affecting the hepatopancreatic, digestive, endocrine, dermal, and nervous systems.

2. Ecology of CyanoHABs —Nutrients

Nutrient and hydrologic conditions strongly influence the dynamics of harmful planktonic and benthic cyanobacteria harmful algae bloom (cyanoHAB) in aquatic ecosystems ranging from streams and lakes to coastal ecosystems. Urbanization, agricultural and industrial development have led to increased nitrogen (N) and phosphorus (P) discharge, which affect cyanoHAB potentials of receiving waters. The amounts, proportions and chemical composition of N and P sources can influence the composition, magnitude and duration of blooms. This, in turn, has ramifications for food web dynamics (toxic or inedible cyanoHABs), nutrient and oxygen cycling and nutrient budgets. Some cyanoHABs are capable of N_2fixation, a process that can influence N availability and budgets. Certain invasive N_2fixing taxa (e.g., *Cylindrospermopsis*, *Lyngbya*) also effectively compete for fixed nitrogen during spring, nitrogen enriched runoff periods, while they use N_2fixation to supplant their nitrogen needs during nitrogen depleted summer months. Control of these taxa is strongly dependent on P supply. However, additional factors, such as Redfield ratio (molar N:P supply), organic matter availability, light attenuation, freshwater discharge, flushing rates (residence time) and water column stability play interactive roles in determining cyanoHAB composition (i.e. N_2fixing vs. non-N_2fixing taxa) and biomass. Bloom potentials of nutrient-impacted waters are sensitive to water residence (or flushing) time, temperatures (preference for >15° Celsius), vertical mixing and turbidity. These physical forcing features can control absolute growth rates of bloom taxa. Human activities may affect “bottom up” physical-chemical modulators either directly, by controlling hydrologic, nutrient, sediment and toxic discharges, or indirectly, by influencing climate. Control and management of cyanobacterial and other phytoplankton blooms invariably include nutrient input constraints, most often focused on N and/or P. While single nutrient input constraints may be effective in some water bodies, dual N and P input reductions are usually required for effective long-term control and management of blooms. In some systems where hydrologic manipulations (i.e., plentiful water supplies) are possible, reducing the water residence time by flushing and artificial mixing (along with nutrient input constraints) can be effective alternatives. Blooms that are not readily consumed and transferred up the food web will form a relatively large proportion of sedimented organic matter. This, in turn, will exacerbate sediment oxygen demand, and enhance the potential for oxygen depletion and release of nutrients back to the water column. This scenario is particularly problematic in long-residence time (i.e., months) systems, where blooms may exert a strong positive feedback on future events (Paerl 2005, Paerl and Otten, 2013).

The impact of phosphorus loadings to the Great Lakes is once again an issue for the Great Lakes-St. Lawrence River ecosystem. Alga blooms fed by excessive

phosphorus from various non-point and point sources are occurring in each of the Great Lakes, but especially Lake Erie, Saginaw Bay on Lake Huron, Green Bay on Lake Michigan and nearshore areas of Lake Ontario. In western Lake Erie the reemergence of cyanoHABs in recent years has been especially troubling, coming after nearly two decades of little or no occurrence of these blooms. As a result of this trend, the Great Lakes Commission adopted a resolution, *Nutrient Management in the Great Lakes-St. Lawrence River Basin*, on October 12, 2011. A report detailing priorities for reducing phosphorus loading in the Great Lakes-St. Lawrence River Basin was published in 2012 (Report of the Phosphorus Reduction Task Force to the Great Lakes Commission September 2012).

3. Cyanotoxins — Chemistry, Toxicology and Production

Cyanobacterial toxins—the cyanotoxins—are bioactive compounds produced by many cyanobacteria from all the major morphological groups**:** unicells, filaments (both branched and unbranched) and both colonial and non-colonial. These potent toxins are responsible, worldwide, for illness and death of wild and domestic animals (aquatic and terrestrial), plus human contact irritations, gastrointestinal distress, and acute, chronic and lethal poisonings. Methods for monitoring, detection and analysis of cyanotoxins have generally followed the same sequence as those for other natural product toxins, especially other marine phycotoxins and mycotoxins. These include:

- bioassays, using small animal, microbial and cell receptor cultures;
- biochemical assays, ranging from immunoassay and enzyme assay to gene-based polymerase chain reaction;
- analytical methods, based on column chromatography, but increasingly mass spectrometry linked to column chromatography (Meriluoto and Codd 2005, Carmichael 2013).

a. Peptides

Most human and animal poisoning by cyanobacteria involves acute hepatotoxicosis caused by a structurally similar group of small molecular weight cyclic hepta- and penta- peptides referred to as microcystins (MCYSTs) and nodularins (NODLNs), respectively (Carmichael 1997, Hudnell 2005, Sarma, 2013a). Over ninety MCYST variants are now described. MCYSTs are also produced by *Microcystis viridis*, *Anabaena flos-aquae*, *Oscillatoria agardhii*, *Nostoc sp.*, *Aphanocapsa cumulus* and *Oscillatoria tenuis*. These cyclic heptapeptides are composed of five common amino acids plus a pair of L-amino acids where most of the differences occur among the ninety variants. Figure 2 illustrates the most prevalent of these peptides called microcrystal-LR (leucine/arginine). Of the peptide toxin-producing genera, *Microcystis* is the main genus found worldwide, and of the three toxic species identified to date, i.e. *M. aeruginosa, M. viridis,* and *M. wesenbergii*, only *M. aeruginosa* has been used in toxicosis studies. It is also the dominant toxic cyanobacteria in the Great Lakes and surrounding areas.

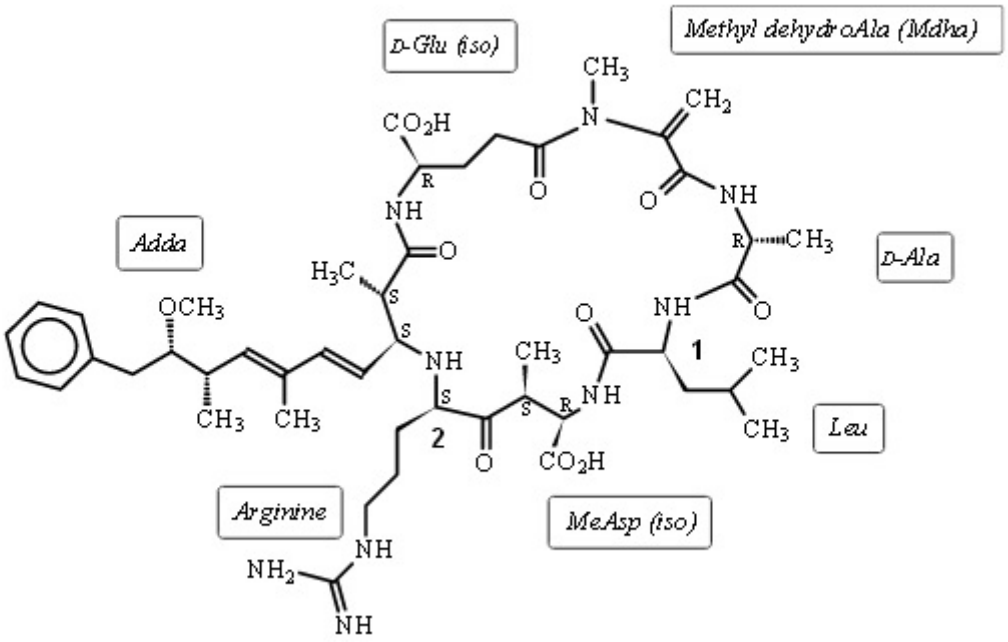


Figure 2. The structure of Microcystin-LR.

Variations occur primarily at positions 1 and 2. For example, microcystin-LR contains the amino acids leucine (L) and arginine (R) at positions 1 and 2 respectively; microcystin-RR has arginine at both positions. Nodularins are similar with the five amino acids Adda-γGlu-Mdhb-βMeAsp-Arg making up the core ring system (Harada, 1996).

Other non-cyclic peptides and amino acids produced by cyanobacteria can have bioactive (pharmacologic) activity. One recently investigated amino acid with neurologic degenerative activity is BMAA (ß-methylamino alanine) (Figure 3). BMAA, originally isolated from a Guam cycad, *Cycas circinalis*, has been proposed as the causative agent of amyotrophic lateral sclerosis or Parkinsonism dementia (Murch et al. 2004). Symbiotic *Nostoc* species associated with the roots of cycads produce BMAA and it accumulates in the seeds of the plant. The indigenous Chamorro people of Guam feed on the flying foxes, which consume these seeds, and are subject to a classic biomagnification of the toxin up through the higher trophic levels (Cox et al. 2005). BMAA may also be incorporated into peptides or proteins, both increasing its biomagnification and acting as a reservoir to slowly releasing toxins upon peptide hydrolysis (Murch et al. 2004).

Cox et al. (2005)surveyed cyanobacteria obtained from culture collections that spanned the entire spectrum of cyanobacterial morphology and taxonomy for the presence of BMAA. BMAA was common in *Nostoc* species isolated from host plants, with 7 out of 10 isolates containing high levels of either the free or protein-bound amino acid, it is ubiquitous in its distribution, being found in freshwater, marine, brackish and terrestrial environments. In free-living cyanobacteria isolated from aquatic

environments, BMAA was present in 95% (20/21) of the genera tested, and in 97% (29/30) of the species tested. Algal strains in culture often produce much lower levels of toxins than found *in situ* suggesting that there is potential for widespread human exposure to this toxin (Cox et al. 2005). BMAA was recently detected in the brains of nine Canadian Alzheimer patients, but was not found in the brains of 14 other Canadians that died from causes unrelated to neuro-degeneration (Murch et al. 2004). Since cycads are not part of the normal Canadian flora, cyanobacteria may be the source of BMAA in these patients. Drinking water contaminated by cyanobacterial blooms has been suggested as a potential pathway for human exposure to BMAA, and a topic which should receive further research.

In a review of human health impacts from several prominent bacteria, cyanobacteria and marine algae, Bienfang et al (2011) summarized the health implications of cyanobacteria-derived BMAA as follows: “Since the initial report of the widespread distribution of BMAA in representatives of all five cyanobacteria morphotypes by Cox et al. [2005], there have been a number of conflicting studies published regarding the detection and quantification of BMAA in cyanobacteria (including blue-green algae nutritional supplements).” Some studies report the presence of BMAA in cyanobacteria using liquid chromatography/mass spectrometry (LC/MS), Liquid chromatography-tandem mass spectrometry (LC/MS/MS) and gas chromatography/mass spectrometry (GC/MS), while others using these methods do not. These authors go on to write: “These disparate findings are likely caused by analyte misidentification and/or differences in methodological sensitivities. Because of potential coelution artifacts, we recommend that that BMAA identification and quantification be based on the LC/MS analysis of BMAA-specific fragments (*m/z* 88) or hydroxysuccinimidyl carbamate (AQC)-derivatized BMAA (*m/z* 258) in order to minimize the possibility of reporting false positive data.” These authors conclude: “Finally, we recommend that future studies should monitor BMAA concentrations using only BMAA-specific LC/MS methods in animals used for human consumption that either directly consume cyanobacteria or forage on plants or prey that may have accumulated cyanobacteria-produced BMAA.”

There have been no studies of BMAA from the Great Lakes area. In view of the absence of studies for the Great Lakes, and the conflicting studies about the occurrence of BMAA in cyanobacteria, BMAA is not a significant risk for the Great Lakes at this time. However, this should not exclude more studies to determine its possible presence in Great Lakes area cyanobacteria.

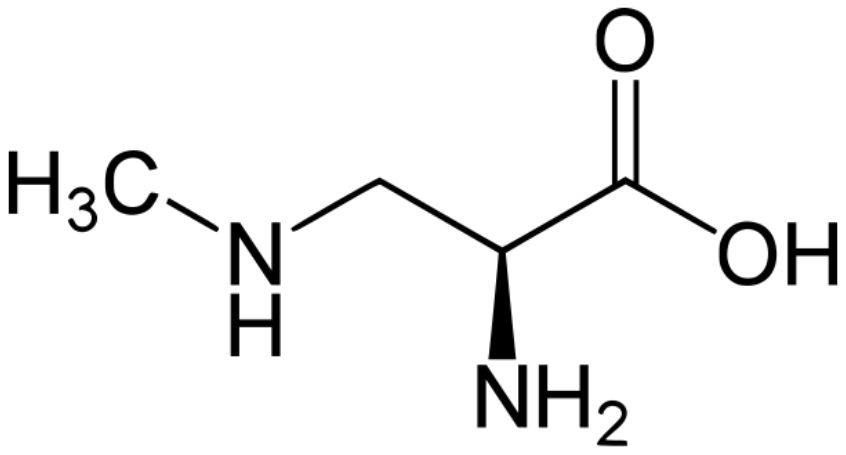


Figure 3. ß N-methylamino-L-alanine (BMAA) M.W. 118

b. Alkaloids

Produced by strains of *Anabaena* and *Oscillatoria (Planktothrix)*, the alkaloid neurotoxin anatoxin-a (antx-a) is a potent post-synaptic depolarizing neuromuscular blocking agent (Figure 4.b). This toxin causes death within minutes to a few hours depending on the species, the amount of toxin ingested, and the amount of food in the stomach. Clinical signs of antx-a poisoning follow a progression of muscle fasciculations, decreased movement, abdominal breathing, cyanosis, convulsions and death. In addition, opisthotonos (rigid "s"-shaped neck) is observed in avian species. In smaller laboratory animals, death is often preceded by leaping movements, while in field cases, larger animals collapse and sudden death is observed. No known therapy exists for antx-a, although respiratory support may allow sufficient time for detoxification to occur followed by recovery of respiratory control. Anatoxin-a was originally isolated from a Canadian isolate of *Anabaena flos-aquae* (Devlin et al. 1977), but is also reported in *A. planktonica*, *Oscillatoria* spp., *Planktothrix* spp., and *Cylindrospermum*. Homoanatoxin-a, a toxic homologue with a propyl group replacing the acetyl group, was isolated from *Phormidium* (*Oscillatoria*) *formosa*.

Another alkaloid cyanotoxin is the cholinesterase inhibitor termed anatoxin-a(s) (antx-a(s)), where (s) means salivation factor. Antx-a(s) is a guanidinium methyl phosphate ester (molecular weight 252) (Figure 4.c). Antx-a(s) is very toxic (i.p. mouse LD_{50} 20 µg kg^{-1}) but is somewhat unstable and becomes inactivated with elevated temperatures (>40^{o} C) and under alkaline conditions. Toxicosis associated with antx-a(s) has been observed in the field in cases involving dogs, pigs and geese. Clinical signs of antx-a(s) toxicosis in pigs include hypersalivation, mucoid nasal discharge, tremors and fasciculations, ataxia, diarrhea, and recumbency. In ducks, the same symptoms occur, plus regurgitation of algae, dilation of cutaneous vessels in the webbed feet, wing and leg paresis, opisthotonos and clonic seizures prior to death. Clinical signs in mice include lacrimation, viscous mucoid hypersalivation, urination,

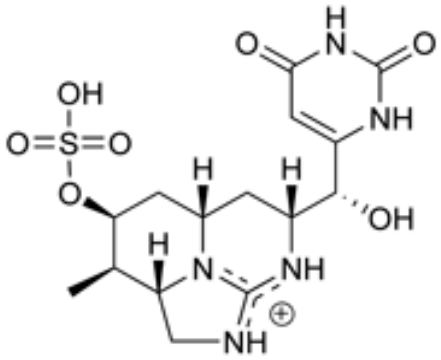
defecation, and death from respiratory arrest. Rats exhibit the same clinical signs plus chromodacryorrhea (red-pigmented "bloody" tears). At the LD_{50}, survival times are 5-30 minutes.

Some strains of *Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya* and *Planktothrix* produce the alkaloid family of neurotoxins referred to as paralytic shellfish toxins (PSTs). The main PSTs found are saxitoxin (Figure 4.d) and neosaxitoxin (Carmichael 1997). These sodium channel blocking agents inhibit transmission of nervous impulses and lead to death in animals by respiratory arrest. Recent research in Australia has shown the widespread occurrence of saxitoxins and related neurotoxins in blooms of *Anabaena circinalis* in rivers and water storage reservoirs (Humpage *et al.* 1993). In addition, it is now known that the freshwater mat-forming cyanobacteria *Lyngbya wollei* can produce saxitoxin analogs. This cyanobacterium is found in several lakes and reservoirs of the southern and south central United States (Carmichael *et al*. 1997, Onodera *et al.* 1997). *Cylindrospermopsis* from Brazil has been shown to produce PSP (Lagos *et al.* 1999). Most recently PSTs have been found in *Lyngbya wollei* from the St Lawrence River in Canada (Lajeunesse et al. 2012). This study found two saxitoxin analogs, LWTX-1 and LWTX-6. The concentration of LWTX-1 was between 209±5 and 279±9 μg^{-1}. No other targeted cyanotoxins, such as anatoxin-a, nodularin, microcystin-LR, microcystin-RR or saxitoxin were found. This demonstrates that cyanotoxins other than microcystins are in the St Lawrence River and in the Great Lakes as well.

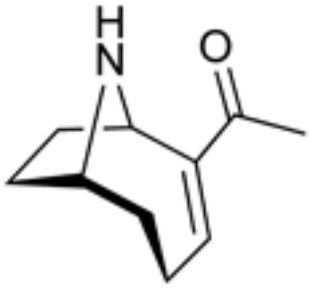
An alkaloid cyanotoxin that does not lead to neurotoxicosis but rather hepato and renal toxicosis is cylindrospermopsin. It is first shown to be produced by the heterocystous *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Raju. It was first implicated in a harmful algal bloom event that occurred in Solomon Dam, Palm Island, North Queensland of Australia in 1979, and which led to severe hepatoenteritis for138 people (Hawkins *et al,* 1985). The structure of cylindrospermopsin (CYN) (MW 416 M+H) was elucidated by Ohtani *et al,* (1992) as a guanidine ester (Figure 4.a). Since then CYN has been found in Anabaena, *Umezakia*, *Aphanizomenon* and *Raphidiopsis*.

Beginning about 1995 *C. raciborski* was identified in eutrophic Florida lakes (Chapman and Schelske 1997). Isolation of this species and examination of its toxin from certain Florida strains has revealed that this species is also producing CYN in at least some Florida lakes (Frey 2004). Since that date *C. raciborski* has been found in ever expanding areas, especially in the Midwest. Holland et al. (2006) documented this expansion and published a paper on studies in Illinois.

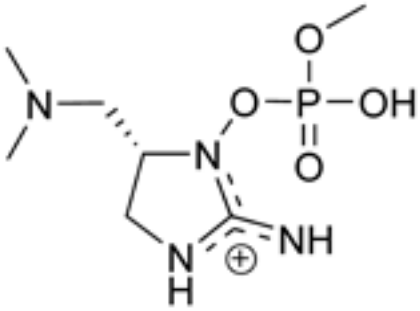
Figure 4. Structures of the Alkaloid Freshwater Cyanotoxins.



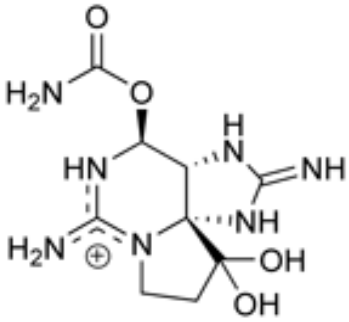
(a) Cylindrospermopsin



(b) Anatoxin-a



(c) Anatoxin-a(s)



(d) Saxitoxin

c. Lipopolysaccharides (LPS)

Weise *et al*. (1970) were the first to isolate LPS (also called endotoxin) from the cyanobacterium *Anacystis nidulans.* LPS are generally found in the outer membrane of the cell wall of gram-negative bacteria, including cyanobacteria. They are an integral part of the cell wall and can elicit irritant and allergenic responses in human and animal tissues that come in contact with the compounds. Cyanobacterial LPS is less potent than LPS from gram-negative bacteria. Although poorly studied, these cell wall components may contribute to human health problems associated with occurrences of cyanoHABs. Some researchers and health officials believe that some water-based cases of contact irritation and gastroenteritis are due to cyanobacteria LPS (J. Hyde, NYS, DOH, personal communication 2013).

Table 1 is a summary of the major cyanotoxins. All cyanotoxins except nodularin, aplysiatoxin, debromoaplysiatoxin and lyngbyatoxin been found in the Great Lakes area.

Table 1. Major Cyanotoxin groups, Toxin-producing Genera and Lethal Dose 50% (LD_{50}).

Toxin or toxin group	Classification by principal target organ systems	Toxin-producing genera	LD_{50}(i.p. mouse)
Microcystins	Hepatotoxins	*Anabaena, Anabaenopsis Aphanocapsa, Arthrospira, Hapalosiphon, Microcystis, Nostoc, Oscillatoria, Planktothrix, Snowella, Synechocystis, Woronichinia*	25->1000 µg/kg
Nodularins	Hepatotoxins	*Nodularia*	30–60 µg/kg
Anatoxin-a, homoanatoxin-a	Neurotoxins	*Anabaena, Aphanizomenon, Arthrospira, Cylindrospermum, Microcystis, Oscillatoria, Phormidium, Planktothrix, Raphidiopsis*	200–375 µg/kg
Anatoxin-a(s)	Neurotoxin	*Anabaena*	20–40 µg/kg
Paralytic Shellfish Toxins (saxitoxins)	Neurotoxins	*Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya, Planktothrix*	10–30 µg/kg
Cylindrospermopsin	General cytotoxin (multiple organ systems affected, including liver, kidney, gastrointestinal tract, heart, spleen, thymus, skin)	*Anabaena, Aphanizomenon, Cylindrospermopsis, Raphidiopsis, Umezakia*	2.1 mg/kg (24 hours) 200 µg/kg (5–6 days)
Aplysiatoxin, debromoaplysiatoxin	Dermal toxins; probable gastro-intestinal inflammatory toxin	*Lyngbya*	107–117 µg/kg
Lyngbyatoxin A	Possible gastro-intestinal inflammatory toxin	*Lyngbya*	250 µg/kg (LD_{100})
BMAA	neurodegenerative	Some to many cyanobacteria	Not determined

B. Occurrence and Distribution of Harmful Algae Blooms(HABs)

1. Worldwide

Marine Harmful Algal Blooms (HABs) are proliferations of microscopic algae that harm the environment by producing toxins that accumulate in shellfish or fish, or through the accumulation of biomass that in turn affects co-occurring organisms and alters food webs in negative ways. Like much of the world's coastlines, nearshore marine waters of the US and Canada have experienced increases in the number, frequency, and type of HABs in recent years (HARRNESS, 2005).

Fresh, brackish and marine Cyanobacteria (blue-green algae) also produce potent toxins that have many of the same health and environmental effects as marine HABs. Animal poisonings from blooms of cyanobacteria in a water supply are part of the published record since 1878 (Francis 1878). A comprehensive reference for the worldwide occurrence and history of toxic cyanobacteria can be found in a monograph by Codd et al. (2006). The most recent books on the topic of toxic cyanobacteria are those supported by the World Health Organization (Chorus and Bartram, 1999) and the US Environmental Protection Agency (Hudnell 2008). The most recent monograph on cyanobacteria that includes a comprehensive review of cyanotoxins is by Sarma (2013).

No reports of marine HAB toxins, other than the Paralytic Shellfish Toxins (PSTs), are known from the Great Lakes areas. Therefore this report will focus on cyanobacteria and their toxins the cyanotoxins.

2. North America

The North American cyanoHAB history is best described by Carmichael and Stukenberg (2006). They report that the North American record of toxic cyanobacterial outbreaks was first characterized by Olson (1960) as, "long before the term algae bloom became a part of the vocabulary of the scientist, primitive man, in looking out over the expanse of blue-green water which constituted his favorite fishing haunt, was probably aware of the fact that notable alterations in the color and clarity of this body of water would occur as the seasons changed. He was conscious of the visual changes, but did not comprehend that they represented an extremely complex interaction between the physical environment and a multitude of free-floating microscopic plants. Even today the exact nature of this relationship is not perfectly understood." This phenomenon was only slightly better understood by the time the first U.S. recorded algal bloom livestock deaths were described in a local South Dakota paper as, "…losses every year unless we keep our stock and poultry away from the lake during the time when the scum is on."

Carmichael and Stukenberg (2006) historical review of cyanoHABs is relied on to present an overview of the pre-2000 United States cyanoHAB occurrences and as a result the pre-1960 original references are not cited. The earliest documented investigation in the United States into the poisonous potential of blue-green algae was recorded in ***The Bulletin of the Minnesota Academy of Science***, though the first written description of an actual outbreak did not come until 1925 when a farmer lost 127

hogs and 4 cows after they drank from Big Stone Lake in South Dakota. He had the lake water analyzed and the livestock deaths were attributed to algae poisoning according to the ***Wilmot Enterprise***. A few years later, ***Cornell Veterinarian*** published a description of five Minnesota cases of algal poisoning.

The first described instance of *human* illness due to algal toxins occurred in Charleston, West Virginia in 1931, and was published in ***The American Journal of Public Health***. A massive *Microcystis* bloom in the Ohio and Potomac Rivers caused intestinal illness in an estimated 5,000 to 8,000 people. According to the article, though the drinking water taken from these rivers was treated by precipitation, filtration and chlorination, these treatments were not sufficient to remove the toxins.

Very few actual algal outbreaks were reported in the next two decades: in the 1930s, only three articles were published on specific algal studies in the United States, and in the 1940s, there were likewise only four articles published, among them a sheep poisoning case in Montana and a case of livestock deaths in North Dakota. Each was described in a separate issue of ***The Journal of the American Veterinary Medicine Association***.

The 1950s showed a marked increase in the number of reported blue-green algae studies in the United States, with a total of nine articles including one outbreak case concerning some domestic animal deaths from algal poisoning in Illinois (***Case History Report from the Illinois State Department of Public Health***).

From the 1960s through the 1970s there were fewer U.S. cases published, five in the 1960s and eight in the 1970s. However, published accounts of algal outbreaks jumped to twelve in the 1980s and 19 in the 1990s (Carmichael and Stukenberg 2006). It should be acknowledged that there is a potential reporting inaccuracy in these statistics, as reporting such events in the United States has always been voluntary. Since 1971, however, in a USEPA and Centers for Disease Control (CDC) cooperative effort, surveillance data from waterborne outbreaks have been more consistently compiled and more conclusively upheld as cyanobacteria-related. A current assessment of cyanoHABs is available from Lopez et al. (2008).

Two surveys of water bodies in the U.S. for cyanotoxins have been done. The first was in 2006 by the United States Geological Survey (USGS): Midwestern US Cyanotoxin Reconnaissance of Cyanobacterial Blooms (2006 USGS MCR). The second was a 2007 EPA National Lake Assessment (U.S. Lakes Microcystin Dataset, 2007). For the USGS 2006 survey, (carried out in August) all lakes (n=23) had detectable microcystins when ELISA (enzyme linked immunosorbent assay) and LC/MS/MS combined, 30% had detectable anatoxin-a, and 9% had detectable nodularin-R (Loftin et al. 2008).

The 2007 EPA funded survey was done in order to develop the U.S. Lakes Microcystin Dataset with relative probable human health effects assessment based on WHO guidelines. Results of this study can be found at: http://water.epa.gov/type/lakes/NLA_data.cfm. This survey looked at microcystin

occurrence in 1150 water bodies. Looking at only microcystins by ELISA, 9 (0.7 %) lakes exceeded WHO recreational guidelines (20 parts per billion (ppb), or 20 micrograms/liter (µg/L)) and 143 (12 %) lakes exceeded WHO drinking water guidelines (1.0 ppb, or 1.0 µg/L) for microcystins (WHO, 2003). Full details and reports of these studies are also available at: http://ks.water.usgs.gov/studies/qw/cyanobacteria/

Some of these water bodies are in the Great Lakes area. For example microcystins were often found in 50 randomly selected lakes in Minnesota as part of this EPA survey (Heiskary and Lindon 2009).

According to a recent review by Graham et al. (2009) at least 22 states in the United States (44 percent) have information available on the Internet about cyanobacteria, cyanotoxins, and potential health risks to humans and animals. At least five states have routine statewide (Iowa, Nebraska, and New Hampshire) or watershed-based (Maryland and Vermont) monitoring programs for cyanotoxins in freshwaters at the state level, and four others(California, Florida, Massachusetts, and Oregon) have developed guidance documents to support monitoring at the local level. Several coastal states (e.g., Florida, Maryland, and Massachusetts) have incorporated the largely freshwater cyanotoxins into existing programs monitoring recreational hazards associated with marine algal toxins.

The situation in Canada is much the same with animal deaths documented since the 1950's with intermittent human gastroenteritis and contact irritations from this same time period. The first published report for Canada was by O'Donoghue and Wilton (1951). In recent years, cyanoHABs have been reported to be a concern for most Canadian provinces, though they remain particularly prominent in the Prairie Provinces (Alberta, Saskatchewan, Manitoba) and in the province of Quebec. A more recent study of Lake of the Woods, Ontario (Chen, et al., 2009) found a maximum concentration of microcystin of 0.69 µg/L for monthly July-September in 2006 and 2007. In 2010, Health Canada analyzed samples from recreational waters, drinking water sources, and treated drinking water based upon unpublished data in four Canadian provinces. Total microcystins were detected in many of the blooms tested, with concentrations in most samples ranging from 0-1.5 µg/L in 71% of 138 samples. A small proportion of samples (4%) had concentrations of total microcystin between 1.5 and 5 µg/L, while 25% of samples had concentrations greater than 5 µg/L and as high as 2000 µg/L. Microcystin was not detected in treated water (Giddings et al. 2012). In a published review of cyanobacterial toxins in Canada by Kodak and Burwell (2007) they also concluded that the occurrence is widespread. Most provinces now have programs that will respond to cyanoHAB events. However it needs to be pointed out that sampling protocol and cyanotoxin detection methods for monitoring are not standardized in Canada or the United States.

Websites for states and provinces in the Great Lakes area having cyanoHAB information are given in Appendix A.

There are currently no national regulations or recommendations addressing cyanotoxins in recreational or drinking water in the United States. In 2004, Health

Canada began the process of updating Guidelines for Canadian Recreational Water Quality. Their working group tasked with developing the guideline assessed approaches used by other jurisdictions worldwide, and concluded that developing guideline values for cyanobacteria (i.e., the cells) and for cyanobacterial toxins (i.e., microcystins) in recreational waters was the preferred approach for the Canadian situation. A guideline value of 20 µg/L for total microcystins in Canadian recreational waters was established using a similar approach to that used in developing the Canadian drinking water quality guideline (Tables 2 and 3; Giddings et al. 2012)

Table 2: Examples of Guidance Values or Standards and Other National Regulations or Recommendations for Managing Cyanotoxins in Drinking Water compared to WHO guidelines.

Country /source document	RMF* required	Cyanotoxins and/ or Cyanobacteria explicitly regulated	S, (P)GV, (P)MAV, (P)MAC or HAL**	Comments; specific action in case of derogation
World Health Organization		Microcystin-LR	1.0 µg/L	Guidelines based on cell concentrations rather than on cyanotoxin concentrations
Canada		Microcystin-LR	*MAC:* 1.5 µg/L	MAC for Microcystin-LR is considered protective against exposure to other microcystins; monitoring frequencies driven by bloom occurrence – more frequent where there is a history of bloom formation
		Anatoxin-a	PMAC: 3.7 µg/L	ATX regulated only in Quebec
United States of America		No national requirements, but action taken by many of the States – see Table 1 in the USA Chapter on p. 139 for the approaches pursued by each of 21 States.(Chorus 2012)		

* Risk management framework (RMF) e.g. WSP, HACCP, PHRMP; **Standard (S); provisional guidance value ((P)GV); provisional maximum value or concentration ((P)MAV / (P)MAC)):; health alert level (HAV)

Table 3: Examples of National Regulations or Recommendations for Managing Cyanotoxins in Waterbodies used for Recreation compared to WHO guidelines.

Country /source document	Management framework required or other comments	Parameter regulated	Values	Actions taken / consequences of derogations
World Health Organization		Microcystin-LR	20.0 µg/L	Guidelines based on cell concentrations rather than on cyanotoxin concentrations
Canada	Bloom risk management programs in some provinces	Microcystin-LR; or Cell Counts	≤20 µg/L ≤100,000 cells/mL	If either of guideline values is exceeded, a swimming advisory may be issued by the responsible authority. Contact with waters where an advisory has been issued should be avoided until the advisory has been rescinded
United States of America	No national requirements, but action taken by many of the States – see Table 1 in the USA Chapter on p. 139 for the approaches pursued by each of 21 States. (Chorus 2012)			

3. Great Lakes Area

The preceding review should make it clear that cyanoHABs and their toxins, the cyanotoxins, are an integral part of water bodies, especially in the summer through fall months throughout the U.S. and Canada. The USGS and United States Environmental Protection Agency (USEPA) surveys of 2006 and 2007 also showed that the greater numbers of cyanoHAB events occurred in the northern Midwest states. This report will focus on whether that situation extends to the Great Lakes area. It will also review the few reports on cases of possible cyanobacterial bloom related human illness and animal death.

Widespread blooms (planktonic and attached, e.g., *Cladophora*) were a recognized impairment in offshore and nearshore areas in the Great Lakes in the 1960s and 1970s. Blooms dominated by the filamentous, non-cyanotoxin producing

cyanobacterium *Aphanizomenon flos-aquae* were also present (Jeff Ruetter, Ohio Sea Grant, personal communication 2013). Concerns at that time were based around impaired aesthetics, taste and odor, food web decline, fouling of beaches, water intakes and fishing nets, and economic impacts. These were addressed largely by targeting total phosphorus to reduce chlorophyll a levels, which was mitigated through reductions in point-source nutrient loadings. In the mid to late 1990's there was a resurgence in algal blooms in parts of the Great Lakes, dominated by cyanobacteria capable of producing toxins or harmful metabolites, such as microcystins, compounds that were unidentified in the 1970s (Watson and Boyer 2009). The first presentation and publication of this reemergence was by Carmichael (2001) and Brittain et al. (2001). These cyanoHABs were dominated by the unicellular colonial microcystin-producing species *Microcystis aeruginosa*. At this same time the invasive zebra mussel (*Dreissena polymorpha*) was receiving attention. Studies by Vanderploeg et al. (2001) provided evidence that zebra mussels promoted blooms of toxic *M. aeruginosa* in Saginaw Bay, western Lake Erie, and other lakes through selective rejection in pseudofeces. Selective rejection depended on "unpalatable" toxic strains of *M. aeruginosa* occurring as large colonies that could be rejected efficiently while small desirable algae were ingested. This work suggested that while phosphorus increases from nonpoint sources would be a major contributor to *Microcystis* blooms, the selective feeding behavior of zebra mussels could also contribute.

Phytoplankton surveys, before this time and since 2001, have shown the increasing trend for toxigenic cyanobacteria in the Great Lakes and surrounding areas (Barbiero and Tuchman 2001, Boyer 2007, Winter et al. 2011).One of the earliest survey studies that showed the increased presence of microcystins was done by Murphy et al. (2003) for Lake Erie. Water samples were collected in the summer of 2001 for microcystin analysis, nutrients and algal enumeration from Hamilton Harbour (Lake Ontario), Wendt Beach (Lake Erie) and Presque Isle (Lake Erie). Microcystin concentrations varied largely. Most levels were below recommended guideline values but some heavy scum found in wind-concentrated areas, were capable of acute poisoning if ingested. This was the case for Hamilton Harbor where the bloom was >90% *Microcystis*, primarily *M. botrys, M. viridis* and some *M. wesenbergii*. At this location, microcystin-RR was the main microcystin, with microcystin-YR and -LR also present. The two samples of August 17 and September 7, taken during the peak of the cyanobacterial bloom, contained 60 and 400 µg/L, respectively of microcystin. A few dying birds were seen in the Hamilton scum. The concentrations of microcystins at the Lake Erie sites were less than 1 µg/L, yet dead birds were common. The authors pointed out that a major limitation with their approach was that current detection (ELISA and HPLC) methods were unable to measure covalently-bound microcystins, the form that is assimilated into the food chain. In another Lake Ontario study Makarewicz et al. (2009) surveyed inshore areas in the United States for microcystins. Within the embayments, ponds, rivers, creeks, shore side, and nearshore and offshore sites of Lake Ontario, microcystin-LR concentrations were low in May, increased through the summer, and reached a peak in September before decreasing in October. Considerable variability in microcystin-LR concentrations existed between and within habitat types within the Lake Ontario ecosystem. Remedial action plans for cyanoHABs were proposed by Watson et al. (2003) for Hamilton Harbor.

In the Great Lakes remote sensing has also been used to demonstrate the increased cyanoHAB trend (Becker et al. 2009). To aid in the detection and occurrence of cyanotoxins, genetic-based methods are also being employed (Rinta-Kanto et at. 2005, Ouellette et al. 2006, Hotto et al 2007), with focus on western Lake Erie and the New York state border of Lake Ontario. PCR assays allow the distribution and abundance of Microcystis to be targeted more directly, and PCR detection of genes required for synthesis of the toxin microcystin provide the greatest specificity. General trends indicate that Microcystis blooms originate nearer to the lake shore with the potential of spreading beyond their point of origin due to surface wind and circulation patterns, leading to a larger area of impact.

In a review of cyanoHABs for the State of the Lakes Ecosystem Conference 2008 (SOLEC), Watson and Boyer (2009) state that Lake Erie channels and embayments are among the most severely impacted areas of the Great Lakes (Table 4).

Table 4. Summary of Cyanotoxin Levels in Lake Erie from 5 Surveys.

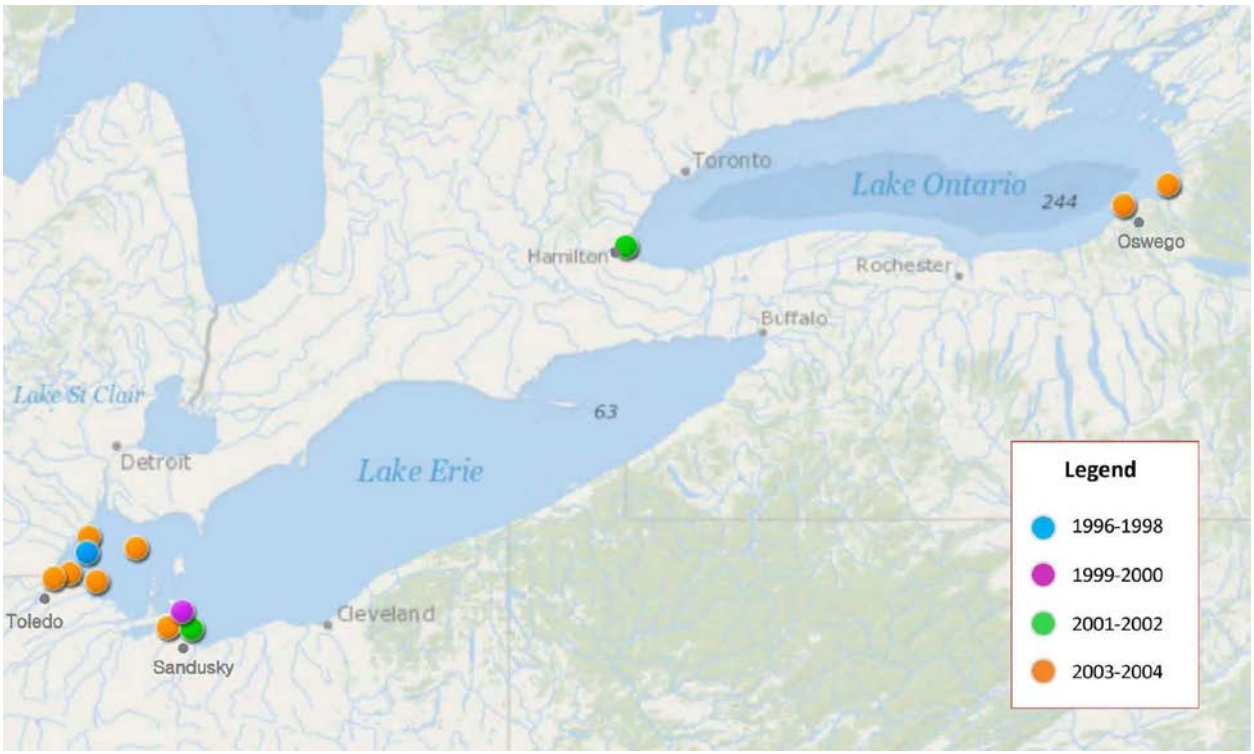
Cruise	Date	# Samples	Toxin	% Samples contain Toxin	Max Level µg/L	Comments
Brittain	Sep-96	44	MC	10	3.4	Western Basin only
MELEE–VII	Jul-02	119	MC	7	0.7	whole lake; highest at Sandusky, Long Pt., Rondeau Bays
			ATX	14	0.04	
			PSTs	0		
MELEE–VIII	Jul-03	59	MC	41	0.65	whole lake; highest in West Basin& Sandusky Bay
			ATX	5	0.11	
Lake Guardian & OSU	Aug-03	48	MC	60	21	West Basin only, highest number Maumee River
			ATX	4	0.2	
MELEE–IX	Jul-04	40	MC	38	>1	Highest number Maumee River Sandusky Bay
			ATX	33	0.6	
			CYL	0		
Limnos	Aug-04	13	MC	85	2.4	Western Basin only
			ATX	31	0.07	
			CYL	15	0.18	
MC=Microcystin; ATX=anatoxin-a; PSTs=saxitoxin + neosaxitoxin; CYL=cylindrospermopsin						

Source: Boyer (2007).

July to October outbreaks of planktonic and benthic taxa show significant interannual, seasonal and spatial variation in origin and impacts. Immense surface blooms (> 20 km^2) have been recorded in the Western basin near the Maumee and Sandusky Rivers, which are potential sources for HABs in Western and West-Central basins. Data from five targeted cruises during 2000-2004 measured a wide range in microcystin levels from detection limits (in 2002) to > 20 µg/L (in 2003). Toxicity and bloom distribution varied spatially and were not restricted to the Western basin. In 2003, highest microcystin concentrations were measured from Maumee Bay, Long Point Bay and Sandusky Harbour. Neurotoxins (anatoxin-a, saxitoxin, neosaxitoxin) and cylindrospermopsin occurred at or near detection limits. In 2001 and 2002, some significant localized microcystin occurrences were also found in the Central and Eastern basins.

A map summarizing locations in the lower Great Lakes where elevated microcystin has been measured, based on references from this section of the report, is shown in Figure 5. Elevated microcystin is interpreted to mean that measured levels of microcystin matched or exceeded the WHO drinking water guideline of 1.0 µg/L for microcystin.

Figure 5. Geographic distribution of high levels of microcystin (> 1.0 µg/L) in the lower Great Lakes between 1996-2004.



a. Case Reports in the Great Lakes Area

Due to the size and diversity of the Great Lakes, the intensity and duration of cyanoHABs is not likely to occur as it does in smaller inland lakes and reservoirs. Their size also means that blooms will occur more inshore, more akin to marine red tide events. The most common cyanotoxin reported in the Great Lakes is microcystin, produced mostly by certain species and strains of the colonial non-nitrogen fixing *Microcystis*. WHO guidance levels for microcystins have been exceeded in open waters for several Great Lakes and other areas. Other cyanotoxins, including anatoxin-a, saxitoxin/neosaxitoxin and cylindrospermopsin have been detected but at levels below any guidance value.

The current status of HABs in the great lakes is given as follows by Watson and Boyer (2011):

Lake Superior – Status: Good

There is very little quantitative current information on HABs in Lake Superior. Severe HAB outbreaks have not been documented in Lake Superior, although cyanobacteria, including *Microcystis*, are detected in samples taken during routine monitoring.

Lake Michigan – Status: Fair

Lake Michigan has a fairly extensive nearshore zone which accounts for a small fraction of the total volume. Yet the nearshore area has a key influence on the lake ecosystem. Cyanobacteria blooms are reported in some coastal regions in eutrophic embayments such as Green Bay and Muskegon Bay.

Lake Huron – Status: Fair

Lake Huron is one of the more oligotrophic of the Great Lakes, yet excessive phytoplankton and potentially toxic HABs occur in some nearshore areas, notably Saginaw Bay and Northern Georgian Bay.

Lake Erie – Status: Fair to Poor

Water levels in Lake Erie typically fluctuate about 36 cm/yr, but in some years up to 50 cm (e.g., 2002). There has been a steep decline in levels from a 1997 peak to below average during recent years, with significant fluctuations due to climate and storm events. This, together with the corresponding dynamics in the physical and chemical regime, has been accompanied by some disturbing trends in biota and system integrity. Not only does Lake Erie have the most extensive nearshore area, but toxic HABs are a particular concern and the focus of several recent studies.

Lake Ontario – Status: Fair

The Lake Ontario Basin has extensive watershed development and urban input. Blooms of cyanobacteria and related impairments (cyanotoxins, taste and odor compounds) have been identified recently in some nearshore areas.

Based upon these status reports (Watson and Boyer 2011), it is expected that

cyanoHAB events and cyanotoxin poisonings might be more prevalent in Lake Erie and to a lesser extent Michigan, Huron and Ontario. One report of anatoxin-a neurotoxicosis was reported among dogs at a small lake in Ontario (Hoff et al. 2007).To locate case reports of cyanobacterial illness and death among animals and humans, the lead author reviewed websites and made contact with persons at the Federal, state and provincial level for all areas adjoining the Great Lakes. Results of this search found that only Lake Erie has probable or suspect cases of human and animal poisoning from cyanoHABs. Case definitions provided by the Ohio Department of Health are as follows:

Case definitions:

Suspect: Exposure to water or to seafood with a confirmed algal bloom AND onset of associated signs and symptoms within a reasonable time after exposure AND without identification of another cause of illness.

Probable: Meets criteria for S*uspect Case* AND there is laboratory documentation of a HAB toxin(s) in the water**.**

Confirmed: Meets criteria for a *Probable Case* combined with professional judgment based on medical review.

For Ohio there were 2 probable and 7 suspect cases of human illness on Lake Erie in 2010. In 2011 and 2012 there were no probable or suspect cases of human illness (Table 5). In 2010 no animal illnesses or death were reported for Lake Erie (Table 6). This is the extent of reports from Ohio provided for this report. No other provinces or states provided any case reports that were documented per prescribed case definitions as confirmed, probable or suspect.

***NOTE:** There is no CLIA-certified laboratory test to confirm the presence of HAB toxins or their metabolites in human blood, tissues, or urine. Until a report meets a case definition, or is determined to be “not a case,” illness reports shall be referred to as “reports of illness” rather than “cases.”

NOTES: Dog Deaths and Bird Illnesses:

*Between July 23 and August 11, Ohio received four reports of dog illnesses associated with Grand Lake St. Marys. The dogs were reported from Mercer County. Three of the four dogs died between July 22 and July 28. Symptoms included: seizures, diarrhea, vomiting and jaundice. Samples from only one dog were collected, but the histopathology was inconclusive due to autolysis (decomposition of the specimen). Stomach contents were negative. Liver and kidney are being held until they conduct their next validation run (date unknown). Until additional testing is conducted, this case is classified as Probable.

On August 3 and August 11, two ill blue herons (Auglaize (1) and Mercer (1)) were reported and referred to the Ohio Department of Natural Resources (ODNR). No conclusion was reached as to what caused the illness of these blue herons.

Table 5. Cyanobacteria Related Human Illness Reports for Ohio Bathing Season 2010-2012.

Body of Water	Toxin Reported?	County of Body of Water	Human Illness, 2010				
			Probable	Suspect	Lost to follow-up	Not a Case	Report Totals
Burr Oak	Yes	Athens	7				7
Grand Lake St. Marys	Yes	Auglaize & Mercer	8		1	12	21
Lake Erie	Yes	Multiple	2	7		1	10
Lake Mac O'Chee (private)	Yes	Logan	19				19
Deer Creek	Yes	Pickaway & Madison	3				3
Berlin Lake Reservoir (US ACE)	No	Mahoning & Portage				1	1
Lake Alma	Yes	Vinton	2				2
Lake Hope	No (bloom only)	Vinton		1			1
Statewide totals			41	8	1	14	64

Body of Water	Bloom Reported?	Toxin Reported?	Human Illness, 2011					
			Out of state	Probable	Suspect	Lost to follow-up	Not a Case	Report Totals
Grand Lake St. Marys	Yes	Yes		1		1	1	3
Buckeye Lake	Yes	Yes		1		1	1	3
Lake Milton	No	No					1	1
Lake Erie	Yes	Yes	1				1	2
Total Statewide			1	2		2	4	9

Body of Water	Bloom Reported?	Toxin Reported?	Human Illness, 2012					
			Out of state	Probable	Suspect	Lost to follow-up	Not a Case	Report Totals
ERIE—Headlands Beach	Yes	NO			1			1
Total Statewide					1			1

Information Courtesy of Laurie M. Billing, Health Assessment Section, Bureau of Environmental Health Ohio Department of Health.

Table 6. Domestic Animal Illnesses Associated with Bodies of Water in Ohio — 2010

Body of Water	Toxin Reported?	County of Body of Water	Domestic Animal Illness				
			Under Investigation	Probable	Suspect	Not a Case	Totals
Grand Lake St. Marys	Yes	Auglaize & Mercer		4*			4
Lake Loramie	Yes (low microcystin)	Shelby		1			1
Burr Oak	Yes	Athens		2			2
Berlin Lake Reservoir (US ACE)	NO	Mahoning & Portage				1	1
East Harbor	Yes (low microcystin)	Ottawa				1**	1
Statewide totals				7		2	9

Information Courtesy of Linda Merchant-Masonbrink Ohio EPA, Division of Surface Water HAB Coordinator-Recreational Waters.

On August 20, Athens County Health Department reported two puppies who died on August 10 and 11 with a 1-2 day onset of lethargy, fever, excessive drooling, vomiting and diarrhea. Dogs displayed clinical signs 1-2 days after exposure to Burr Oak Lake. Both dogs met probable case definition.

On August 26, an owner reported that her 6 yr old boxer was acutely depressed, anorectic and appeared in pain for 3 days after wading and gulping water from the lake at West Branch State Park/Berlin Lake. Dog had repeated exposure August 5-13. Owner also reported illness. However, a cyanobacteria bloom was not confirmed for this body of water during the dates in question, and therefore the canine and the human illness reports were both classified as “not a case.”

**On September 8, a report was received of a Labrador with water exposure at East Harbor, and subsequent decline including hemolytic anemia. ODH/DNR report review showed no record of a reported bloom (or toxin testing) during the period of exposure (07/03/10), and this report was therefore assigned the classification of “not a case.”

C. Human Health Effects from CyanoHABs

1. Human Exposure

As a general statement, acute lethal toxicosis in humans from cyanotoxins, in treated water supplies, should not occur because normal filtration, coagulation and

other treatment processes in municipal water supplies is designed to remove toxic cells and released toxins to levels below that necessary to cause acute lethal effects. However, in cases of heavy cyanobacteria blooms and where the normal water treatment process is inadequate or not properly operated, toxic cells and free toxins have been present in finished drinking water supplies. There are reports, for the United States and Australia, that cyanotoxins have been implicated in human illness (i.e. acute non-lethal or chronic toxicity) from municipal water supplies, especially after the bloom has been treated by copper sulfate to lyse the cells and release more of the toxins into the distribution system. In these and other cases involving accidental ingestion, the symptoms reported include abdominal pain, nausea, vomiting, diarrhea, sore throat, dry cough, headache, blistering of the mouth, atypical pneumonia and elevated liver enzymes in the serum (Chorus and Bartram 1999).

The recreational use of lakes and rivers (oral and dermal route), is a major route of exposure (Stewart, et al., 2011). The consumption of drinking water, use of showers in public and private locations, and from water sports such as waterskiing (dermal and inhalation) is the second most common route of exposure, although no confirmed fatalities are known by this route. In addition, a minor route of potential exposure is from ingestion of cyanobacteria health food tablets (oral route) (Codd, 1999).

Acute and (rarely) acute lethal toxicity have long been shown for cyanotoxins (Carmichael 1981, Chorus and Bartram 1999), especially microcystins. A recent acute toxicity report is from Argentina (Giannuzzi et al. 2011). More recently, chronic toxicity from microcystins in China has been found (Chen et al. 2009, Li et al. 2011). In the latter study, microcystin was detected in most samples of water and aquatic food from two lakes. Children who drank water from the lake with the highest microcystin concentrations had a total estimated daily microcystin intake of 2.03 µg, a value higher than the 1µg/ L level proposed by the World Health Organization. Hepatitis B virus (HBV) infection, use of hepatotoxic medicines, and microcystin exposure were associated with liver damage. Liver enzyme levels for AST and ALP were significantly higher in high-microcystin-exposed children than in low-exposed children and unexposed children, when participants who were HBV-positive or hepatotoxic medicine users were excluded from the analysis. In conclusion, their results suggest that chronic exposure to microcystin may be associated with liver damage in children in the Three Gorges Reservoir Region.

Three epidemiological studies have been done on cyanobacteria in recreational waters (Stewart et al. 2006, Backer et al. 2008, Backer et al. 2010) in the United States. The 2008 study involved a small lake in Michigan and is pertinent to the Great Lakes area. This study did document low levels of microcystins in the water and in aerosol samples. Respiratory viruses were not detected but low levels of *E. coli* were. Yearly duration of exposure is shorter in those countries (i.e. U.S. and Canada) where the bloom growth season is shorter (3-6 months) compared to those with milder climates such as Australia and South Africa (6-12 months). The other two studies also detected microcystins but no toxicity was documented.

In Canada one retrospective epidemiological study was done on four lakes in Quebec (Lévesque, et al., 2014). While not directly relevant to the Great Lakes, it does have relevance for the Great Lakes area. Summary points of that study are:

1) There was a positive relationship between contact with bodies of water and GI symptoms relative to the numbers of cyanobacteria.
2) Symptoms positively correlated with cell numbers include sore muscles, skin irritation, earaches, nausea, diarrhea, stomach ache and vomiting.
3) The authors were inclined to conclude cyanobacteria LPS was correlated with the symptoms.

The only confirmed route of exposure for acute lethal human toxicity from cyanotoxins is from dialysis water used in a medical facility in Brazil. The confirmed outbreak occurred at a dialysis center (termed Clinic A) in Caruaru, Brazil (8°17' S, 35° 58' W), located 134 km from Recife, which is the state capital of Pernambuco. At Clinic A, 116 (89%) of 130 patients experienced visual disturbances, nausea and vomiting following routine hemodialysis treatment between February 13-20, 1996. Subsequently, 100 patients developed acute liver failure and of these 70 died. As of October 1997, 53 of the deaths could be attributed to a common syndrome now called "Caruaru Syndrome". This syndrome included: 1) symptoms: painful severe enlargement of the liver, jaundice and a bleeding diathesis manifested by hemorrhagic spots, bleeding from the nose and methrorrhagia, 2) laboratory picture: elevated transaminases, variable hyperbilirubinemia, prolonged prothrombin time and severe hypertriglyceridemia, 3) histopathology: light microscopy — disruption of liver plates, liver cell deformity, necrosis, apoptosis, cholestasis, cytoplasmic vacuolization, mixed leukocyte infiltration and multinucleated hepatocytes; electron microscopy — intracellular edema, mitochondrial changes, rough and smooth endoplasmic reticulum injuries, lipid vacuoles and residual bodies. This outbreak received a large amount of media and public health authority attention in Brazil and has been reported on in many countries. The history of this outbreak and the history and summary of the epidemiology have been published (Jochimsen *et al*. 1998, Pouria *et al.* 1998). More detailed information on this outbreak provides evidence for the cyanotoxins present and the cyanobacteria that produced them (Carmichael et al. 2001, Azevedo et al. 2002).

Exposure from cyanotoxins can also occur through the food chain as demonstrated by one study from 2006 focused on algal toxin levels in perch in the Western Basin of Lake Erie (Wilson et al. 2008). During the summer of 2006, the western basin of Lake Erie experienced a bloom of the toxigenic cyanobacterium *Microcystis aeruginosa*. Across 11 sites, intracellular, particulate-bound microcystin levels in the seston increased to levels that exceeded World Health Organization guidelines for drinking water exposure (1 µg/L). In contrast, toxin concentrations in yellow perch (*Perca flavescens*) muscle tissue (n = 68) declined from June to August, were negatively related to algal toxin levels, and did not exceed a conservative chronic exposure concentration that was estimated using proposed United States Environmental Protection Agency (US EPA) guidelines. However, microcystin concentrations in yellow perch liver did exceed US EPA chronic exposure guidelines,

were on average 125 times higher than muscle toxin concentrations per unit dry weight, and varied little throughout the summer. Based on current guidelines, humans do not appear to be at risk when consuming the muscle tissue of Lake Erie yellow perch collected during large-scale cyanobacterial blooms. However, their study highlights the need for a better understanding of the trophic transfer of cyanobacterial toxins through aquatic food webs in diverse ecosystems with an emphasis on understanding if these compounds could accumulate sufficiently to affect human health.

An expanded picture of the emerging human health impact from cyanoHABs begins to appear from survey studies funded by the Centers for Disease Control (CDC). Between 2007 and 2011, 10 states (FL, MD, NC, NY, MA, IA, OR, SC, VA, WI) were funded by CDC to develop and implement activities to address the public health effects from harmful algal blooms. They were required to input data on human illnesses associated with exposure to harmful algal blooms into CDC's Harmful Algal Bloom-related Illness Surveillance System (HABISS). During that time period there were over 450 possible cases of illness associated with HABs. Of those, over 200 were identified as suspect/probable cases of illness associated with HABs, and most were ciguatera fish poisoning. From the original possible cases, over 170 (36%) were associated with a freshwater HAB exposure. However, as with most cases of cyanoHABs, very few were confirmed with positive findings for cyanobacteria/cyanotoxins in relevant water samples. Instead they were classified as probable or suspect (personal communication Dr. Lorrie Backer 2013).

This lack of reporting, which if present would allow for standard reporting of case classification, could well be corrected beginning in 2013. Funding has been created for a HABs module within CDC's National Outbreak Reporting System (NORS). It will not be the standard outbreak reporting format, but will be tailored to include environmental data including animal poisonings. Since the majority of freshwater and marine HAB poisonings are wild and domestic animals this format will allow a more accurate reporting of HAB toxicities. The long-range plan is to put everything from HABISS into NORS once it is built (personal communication Dr. Lorrie Backer 2013).

2. Risk Associated From Exposure to CyanoHABs

Toxic cyanobacteria are now recognized as the group of HAB organisms that are primarily responsible for HAB related events in fresh and brackish waters. However, it is difficult to assign reliable estimates of health risk from cyanoHABs exposure since risk requires an estimate of the expected frequency of undesirable effects caused by exposure to these toxicants. Estimation of risk involves establishing relationships between actual exposures and the expected response. While research is slowly accumulating that will give suitable numbers for estimating risk from toxic cyanobacteria, the term risk should be used in discussion of cyanotoxins only when quantities of toxic cells are available, warranting use of the term risk. Otherwise, the term hazard should be used to designate a general threat from exposure to cyanotoxins. The most likely route for humans to be exposed to cyanotoxins is via drinking water, medical dialyses, recreational waters or consumption of cyanobacterial health food products (Gilroy et al. 2000). For a discussion of human risk and exposure

to cyanotoxins see Carmichael (2001), Ressom et al. (1994), Chorus and Bartram (1999), Hitzfeld et al. (2000), Codd et al. (2005), Orr and Schaeider (2006), Funeri and Testai (2008), Poste et al. (2011) and Chorus (2012).

Another important factor in assessing risk of the cyanotoxins, especially the hepatotoxins is that the microcystins and nodularins are potent tumor promoters through their inhibition of protein phosphatases (Falconer and Humpage 1996). This added risk factor (i.e. tumor promotion) may become important in factoring into calculations of risk, along with data obtained from animal exposures and other toxicological and chemical data (Zegura et al. 2011). Indirect evidence supporting tumor promotion and human liver cancers from MCYST exposure comes from the studies of Yu (1989) in China, and Svirˇcev et al. (2009) in Serbia. In 2006 the International Agency for Research on Cancer (IARC) reviewed current evidence for carcinogenic potential of microcystin-LR. Members of the IARC Working Group reached the following consensus during its review:

1) There is inadequate evidence in humans for the carcinogenicity of microcystin-LR.
2) There is inadequate evidence in experimental animals for the carcinogenicity of microcystin-LR.
3) There is strong evidence of mechanistic data for the carcinogenicity of microcystin-LR.
4) Microcystin-LR was therefore classified to group 2B, indicating that it is possibly carcinogenic to humans. Source: http://oncology.thelancet.com Vol. 7 August 2006 (Grosse et al. 2006)

One preliminary epidemiological study has been done on cancer risk from cyanotoxins in drinking water from Grand Lake St. Marys (GLSM) in Ohio. In this observational study, the city of Celina (Mercer County) Ohio that had a periodic contaminated surface water supply, had its cancer incidence compared with two control cities, St. Marys, and Wapakoneta (Auglaize County) in Ohio, both served by ground water. Results found inconclusive evidence to support that cyanotoxins from GLSM are associated with excess risk of cancer from drinking water (Soward 2011).

a. Bacteria and Cyanobacteria Like Bodies (CLB) in Humans

In 1989 and 1990 CDC reports described the detection of Cyanobacteria-Like Bodies (CLB) in the stools of persons with a prolonged syndrome of diarrhea, anorexia, and fatigue (CDC MMWR 1991, Long et al. 1991). In each of these reports, affected persons either were immunocompromised or had recently traveled to tropical countries. During 1989 and 1990, the first three reported outbreaks of this CLB-associated syndrome occurred in immunocompetent populations, affecting at least 150 persons. That report summarized investigations of the outbreaks, which occurred in Chicago in 1990 and in Kathmandu, Nepal, in 1989 and 1990. The Chicago outbreak has relevance for the Great Lakes and cyanobacteria.

On July 9, 1990, the infectious diseases department at a hospital in Chicago was notified that several staff physicians had onset of diarrhea on July 7-8. In general, manifestations included a 1-day prodrome of malaise and low-grade fever, followed by explosive watery diarrhea, anorexia, severe abdominal cramping, nausea, and occasional vomiting. Remission of diarrhea usually occurred after 3-4 days, but was followed by a cycle of relapses and remissions lasting up to 4 weeks. During remissions, patients noted continued malaise and anorexia, sometimes accompanied by constipation. From July 10 through August 7, stool specimens were obtained from 20 ill persons (17 house staff physicians and three other employees). Cultures were negative for *Salmonella, Shigella, Campylobacter, Yersinia,* and *Vibrio*, and ova and parasites were not detected. However, direct and acid-fast stain microscopic examination of stool specimens from nine of the house staff physicians and one of the other employees revealed the presence of CLB (CDC MMWR 1991, Long et al. 1991).

3. Animal and Plant

It is now well established that cyanotoxins have been responsible for intermittent but repeated outbreaks of wild and domestic animal illness and death for over 100 years. The occurrences are worldwide and include Canadian and United States Great Lakes areas. In addition to animal and human poisonings, exposure of plants to cyanotoxins also carries risk-both for plants and for animals and the humans who consume them. A literature review by Babica et al. (2006) indicated that plants are generally not killed by environmentally relevant levels of cyanotoxins but are growth inhibited, which lowers crop yields and increases the risk of human exposure via the consumption of exposed plants. Fifteen common crop plants were exposed to microcystin-LR and accumulation was determined. Results do indicate some plant stress as well as some bioaccumulation (Milligan 2009). In a recent study by Hereman and Bittencourt-Olivera (2012) lettuce leaves sprayed with microcystins at concentrations of 0.62-12.5 µg/L accumulated concentrations of approximately 8-177 µg/Kg. This indicates that plants can accumulate microcystins that exceed 2 µg/day, the daily intake limit set by the WHO for drinking water.

4. Summary of CyanoHAB Health Effects in the Great Lakes Area

This report provides more background and supporting material on the risk from cyanoHABs to North American freshwater supplies than it does for documenting human health cases relative to the Great Lakes. What we now know is that cyanoHABs have been occurring at least since European settlement in North America. Their frequency, intensity and duration have increased beginning post World War II with their greatest increase in the 1970's. Basic research during this time period now allows us to understand the bioactive compounds and toxins they produce and begin to document human and animal poisonings, plus economic losses. This documentation shows that cyanoHABs occur in all regions of North America but are most prevalent in the Midwest where agriculture and development have altered the environment most. This includes man-made dugouts, ponds and reservoirs plus natural lakes, ponds and rivers where development has been greatest and environmental planning less. Weather and climate variations also influence cyanoHABs. CyanoHABs have occurred in all states and

provinces in the Great Lakes area, with several regions reporting animal illness and death, primarily dogs and livestock. The Great Lakes, due to their size, water mass and lesser degree of eutrophication, restrict cyanoHABs to inshore areas and to the more nutrient rich areas of a lake. For this reason only Lake Erie currently experiences cyanoHABs and cyanotoxins with probable cases of human illness. All other Great Lakes do experience intermittent and periodic cyanoHABs with documented levels of certain cyanotoxins, but no confirmed or probable human illness or toxicity has been reported. The dominant toxigenic cyanobacterium found in the Great Lakes is the colonial, non-nitrogen fixing genus *Microcystis. Microcystis* is only known to produce microcystins but since other cyanotoxins have been detected, including anatoxin-a, cylindrospermopsin and paralytic shellfish toxins (PSTs or saxitoxins), we need to conclude other cyanobacteria are present and producing cyanotoxins. These are most likely in the genus *Anabaena, Aphanizomenon* and *Planktothrix*, but other cyanotoxin producers should not be ruled out. Clearly more research is needed to understand the dynamics of cyanoHABs and health risk in the Great Lakes.

Since our historical context for understanding cyanoHABs and their toxins is only about 30 years old and only about 20 years old in the Great Lakes, we should not take the low number of confirmed cases of human illness directly resulting from exposure to cyanoHABs or cyanotoxins to mean that cyanoHABs and cyanotoxins have negligible risk or impact upon human health. In fact, there are numerous examples in infectious disease and toxicology where confirmed cases of human toxicity have been scant, but the widespread importance of protecting humans from exposures or minimizing the prevalence of the agent in question was well appreciated. One current example is norovirus, where the pathogen lacks an in- vitro cell-culture system for human diagnosis, but is estimated to cause up to 15% of acute gastroenteritis in people of all ages and is a focus of vaccine research and prevention guidelines (MMWR 2011, Lee 2013). With this appreciation and the dedication of resources to further study of HAB effects in humans, the true threat to human health can be learned, and the population appropriately informed and protected.

One need look no further than the same IARC Group 2B carcinogens list that Microcystin-LR is on to find another example of this. Group 2B identifies those compounds that are possibly carcinogenic to humans. Aflatoxins are produced by the fungus Aspergillus, that grows on grains and other crops, which means these toxins can readily find their way into human and animal foods. M_1 is an epoxide metabolite of aflatoxin B_1 that has long been considered to be one of the most potent naturally occurring liver carcinogens. M_1 is produced by animals that ingest feed contaminated with aflatoxin B_1, and it was learned that M_1 can find its way into milk and dairy products, including infant formula. When this was realized increased efforts and resources to study its carcinogenicity led to the understanding that M_1, like its precursor B_1, is in fact a group 1 human carcinogen. *Originally*, however, M_1 was placed on the Group 2B list. The parallels between Microcystin-LR and aflatoxin M_1 are intriguing with respect to Group 2B placement: synergistic effects when ingested by people infected with Hepatitis B, strong mechanistic understanding of the carcinogenic potential (M_1 affects the tumor suppressor *p53*), yet limited toxicological data in humans or animals at the time of its assessment for group 2 or 1 placement (Anfossi et al. 2011).

The increased exposure risks of humans to M_1 are what prompted their increased study. The effects of global warming and a rise in the incidences of HABs are likewise leading to increased exposures of people to cyanotoxins, especially in recreational waters. Hopefully, this realization will lead to a concomitant increased support for toxicological studies of cyanobacterial toxins, so that the real risks to human health, not just as carcinogens but also as gastrointestinal irritants and dermatoxins, can be better assessed.

D. CyanoHAB Indicators and Evaluation of Health Risk

Indicators are designed to

i) Educate/influence policy and decision on status and trends;
ii) Improve data accessibility;
iii) Measure success;
iv) Foster 'adaptive management', watershed level ecological health assessment, and support for additional remedial/ preventive management;
v) Promote stewardship through public outreach (Watson and Boyer 2011).

Well-designed and carefully selected indicators serve as practical, economical and responsive tools for tracking ecosystem changes. Indicators are specific, well-defined, and measurable variables that reflect some key characteristic that can be tracked through time to signal what is happening within and across ecosystems. The Heinz Center State of the Nation's Ecosystems 2008 report uses several categories of indicators in coming up with its 108 indicators of the nation's health (The State of the Nations Ecosystems 2008). Indicator categories important for this Great Lakes report include: Biological—which includes the indicator harmful algal events, and Chemical—which includes the indicator nutrients nitrogen and phosphorus. Harmful algal bloom events are defined as an increased abundance of algae species that can cause direct damage to animal tissues or illness and death among humans and animals. While a specific list of the cyanoHAB species in the Great Lakes has not been done, it seems they could include most of the ones in the toxigenic cyanobacteria key in Chapman (2010). While the Heinz report uses the term harmful algae event, this report will consider that term synonymous with HABs. Ideally, the number of HABs should be quantified on some basis, i.e. low, medium and high intensity for marine coastal waters and freshwater bodies. However there is no continent or nationwide monitoring or reporting program for HABs, nor are there generally accepted definitions of low, medium, and high density. In addition, there are important indicators for HAB health risk. These would be genera and species of HABs plus the toxins and levels produced. In an attempt to make some semi-quantitative conclusions about HAB risk for the Great Lakes and transboundary waters, this report relied on data available for nutrient trends, HAB events, species present and cyanotoxins identified.

Since the United States has no federal regulations defining acceptable levels of cyanotoxins in drinking or recreational waters (Tables 2 and 3), most states rely on guidelines published by the WHO or on risk assessments based on the WHO data to manage these water bodies. However, these guidelines were based on cell concentrations rather than on cyanotoxin concentrations, and not all cyanobacterial blooms produce toxins. Resource managers and public health officials are often left

with the difficult choice between protecting public health by closing a water body with a significant algal bloom that may not be producing toxins, or protecting the local tourism economy by keeping a water body with a visible, aesthetically unappealing bloom open for use. In addition to using the WHO guidance, some states have done their own risk assessments to develop guidelines to support public health decision making, such as posting advisories or closing water bodies. For example, the Office of Environmental Health Hazard Assessment of the California Environmental Protection Agency issued guidance on six cyanotoxins in 2012 (Butler et al. 2012).The guidance provides calculated action levels that may be applied by local, regional, state, or tribal entities to reduce or eliminate exposure of people and animals to algal toxins. Oklahoma was the first state in the U.S. to pass legislation limiting exposure to freshwater algae (the bill can be viewed at: http://legiscan.com/gaits/view/366438). The new law requires the Oklahoma Tourism and Recreation Department to maintain a public website (see www.checkmyoklake.com) that provides information about cyanoHABs to the public, including monitoring data collected by the Oklahoma Department of Environmental Quality, the U.S. Army Corps of Engineers, and municipal authorities. It also requires any agency with authority to manage recreational waters to post signs directing people to the website for information. The legislation also formalizes the responsibility for warning people about cyanobacteria and cyanoHABs blooms and sets the health related warning thresholds: tourism officials will warn lake users if algae cell counts exceed 100,000 cells/mL and microcystin concentrations exceed 20 µg/L (Backer 2012). Since legislation is established on algae cell counts and microcystin concentrations, either of these measures could be used as an indicator of pathogen risk.

Due to these programs and legislation, we are slowly becoming better able to assess risk for cyanoHABs now that the appropriate indicators are being defined. This makes it possible to employ a risk assessment matrix for cyanoHABs. For example using the WHO action level of 100,000 cells/ml (for a toxigenic species — Table 1), blooms exceeding this level should have their source water closed to recreational use until cell levels fall below this level. In a more formal risk assessment, it is also important to know if the toxigenic bloom is producing one or more cyanotoxins, and what the concentrations are. It is now established that blooms can have highly variable levels of cyanotoxins, ranging from non-detect to thousands of micrograms per liter. Most states and provinces that the author has worked with set up their initial monitoring and management plans to respond to cell counts once a toxigenic species is identified. This has worked well for initial establishment of occurrence and distribution of cyanoHAB events. However, over time it has become clear that there are an increasing number of events and that it may be too conservative to place warnings on closures for all of these events based upon cell counts. The economic costs involved from recreational, fishing and increased water treatment may be unwarranted in some cases. This places renewed importance on having rapid, sensitive, established analytical, immunological and genetic tests available (Carmichael 2013, Meriluoto and Codd 2005, Sivonen 2005, Rasmussen et al. 2008, Fortin et al., 2010). An example of this risk process in Illinois is given by Holland et al. (2006). This would be the recommended approach for the Great Lakes as cyanoHAB programs move forward.

Canada has adopted guidelines for microcystins in drinking and recreational waters. In 2002, Health Canada approved a drinking water guideline for microcystin-LR which was deemed protective of total microcystins. More recently (2012), a guideline for recreational water (microcystin toxin and total cells) was approved.

Health Canada's 2002 risk assessment review classified microcystin-LR as "possibly carcinogenic to humans" placing it in Group IIIB (inadequate data in humans, limited evidence in experimental animals). For compounds in Group IIIB, the lowest-observed-adverse-effect level (LOAEL) or no-observed-adverse-effect level (NOAEL) from the most suitable chronic or sub-chronic study is divided by an appropriate uncertainty factor, to derive a tolerable daily intake (TDI). Such an approach was used for microcystin-LR, which remains the only cyanotoxin for which there is sufficient information available to derive a guideline value.

A TDI of 0.04 µg/kg body weight per day was derived from a NOAEL of 40 µg/kg body weight per day for liver changes in a 13-week mouse study (Fawell et al., 1994), using an uncertainty factor of 1000 (x10 for intraspecies variation, x10 for interspecies variation and x10 for the less-than-lifetime study).

A maximum acceptable concentration (MAC) of 0.0015 mg/L (1.5 µg/L) for microcystin-LR was calculated from the TDI by assuming a 70-kg adult consuming 1.5 L of water per day, as well as allocating 80% of the total daily intake to drinking water(the major route of exposure to these toxins is via drinking water). Although the MAC is derived for microcystin-LR, it is considered to be protective against exposure to other microcystins (total microcystins, i.e., free plus cell bound) that may also be present.

E. Economic Effects

Economic losses from cyanoHAB blooms in the Great Lakes area have not been well documented. In some cases people have made verbal estimated losses to tourism (recreation, fishing etc.), increased water treatment costs, decreased property values and losses from environmental degradation. An example was the loss from operation of approximately 100 licensed charter boats (out of 800) due to the extensive cyanobacteria blooms on Lake Erie in 2011 (Rick Unger, personal communication to Jennifer Boehme). It should not be unreasonable to estimate that individual cyanoHAB events on Lake Erie cause a few million dollars in economic losses.

Economic costs from eutrophication and harmful algal blooms have only recently been estimated. Dodds et al. (2009) compared current total nitrogen (TN) and total phosphorus (TP) concentrations for the USEPA nutrient ecoregions with estimated reference conditions. In all nutrient ecoregions, current median TN and TP values for rivers and lakes exceeded reference median values. In 12 of 14 ecoregions, over 90% of rivers currently exceed reference median values. They calculated potential annual value losses in recreational water usage, waterfront real estate, spending on recovery of threatened and endangered species, and drinking water. The combined costs were approximately $2.2 billion annually as a result of eutrophication in U.S. freshwaters. The greatest economic losses were attributed to lakefront property values ($0.3-2.8

billion per year, although this number was poorly constrained) and recreational use ($0.37-1.16 billion per year). They state their evaluation likely underestimates economic losses incurred from freshwater eutrophication. They documented potential costs to identify where restoring natural nutrient regimes could have the greatest economic benefit. And their research exposed gaps in current records (e.g., accounting for frequency of algal blooms and fish kills) and suggests further research is necessary to refine cost estimates.

Steffensen (2007) provided estimates on economic losses for several case studies from Australian rivers and water storage facilities from the late 1980's through the 1990s. Economic losses considered the following costs:

- Those associated with recreation and tourism such as accommodation, transport and tourism;
- Those associated with commercial recreation facilities such as caravan and tourist parks;
- Those associated with the amenity value including aesthetics; and long-term costs related to permanent loss of trade.

Australian Dollar losses for three cases totaled about $10 million. Total estimated costs for this time period were 180-240 million per year but costs have increased considerably since then. It would not be an understatement to write that direct costs are now in the billions of dollars per year. Similar arguments could be made for all developed countries.

F. References

Anfossi,L., Baggiani, C., Giovannoli, C., and Giraudi, G. 2011. Occurrence of Aflatoxin M1 in Dairy Products, Aflatoxins - Detection, Measurement and Control, Dr Irineo Torres-Pacheco (Ed.), ISBN: 978-953-307-711-6, InTech. http://www.intechopen.com/books/aflatoxins-detectionmeasurement-and-control/occurrence-of-aflatoxin-m1-in-dairy-products

Azevedo, S.M.F.O.,Carmichael, W.W., Jochimsen, E.M., Rinehart, K.L., Lau, S., Shaw, G.R., and Eaglesham, G.K. 2002. Human intoxication by microcystins during renal dialysis treatment in Caruaru-Brazil. Toxicology 181: 441-446.

Babica, P., Blaha, L., and Marsalek, B. 2006 Exploring the Natural Role of Microcystins — A review of effects on photoautotrophic organisms. Journal of Phycology,42(1): p. 9-20.

Backer, L.C. 2012. Freshwater Algal blooms & Public Health. Lake Line. Fall. Pp. 9-12

Backer, L.C.,Carmichael, W.,Kirkpatrick, B., Williams, C., Irvin, M.,Zhou, Y., Johnson, T.B., Nierenberg, K., Hill, V.R., Kieszak, S.M., and Cheng, Y-S. 2008. Recreational Exposure to Low Concentrations of Microcystins During an Algal Bloom in a Small Lake. Marine Drugs. 6:389-406.

Backer, L.C., McNeel, S.V., Barber, T., Kirkpatrick, B., Williams, C., Irvin, M., Zhou, Y., Johnson, T.B., Nierenberg, K., Aubel, M., LePrell, R., Chapman, A., Foss, A., Corum, S., Hill, V.R., Kieszak, S.M. and Cheng, Y-S. 2010. Recreational Exposure to Microcystins During Algal Blooms in Two California Lakes. Toxicon. 55:909-921.

Barbiero R., & Tuchman M. 2001. Results from the U.S. EPA's biological open water surveillance program of the Laurentian Great Lakes: I. Introduction and phytoplankton results. Journal of Great Lakes Research.27**:**134-154.

Becker, R.H., Sultan, M.I., Boyer, G.L., Twiss, M.R., and Konopko, E. 2009. Mapping cyanobacterial blooms in the great lakes using MODIS. J. Great Lakes Res. 35: 447-453.

Bergey's Manual of Systematic Bacteriology. Vol. 3. Sec. 19. Oxygenic Photosynthetic Bacteria. Williams and Wilkins.1989.

Bienfang, P.K.,DeFelice, S.V., Laws, E.A.,Brand, L.E., Bidigare,R.R., Christensen, S., Trapido-Rosenthal, H., Hemscheidt,T.K., McGillicuddy Jr., D.J., Anderson, D.M., Solo-Gabriele, H.M., Boehm,A.B. and Backer, L.C. 2011. Prominent Human Health Impacts fromSeveralMarine Microbes: History, Ecology, and Public Health Implications. Int. J. Microbiol. 2011:1-15.

Boyer, G.L. 2007.Cyanobacterial toxins in New York and the Lower Great Lakes ecosystems. In: Hudnell, H.K. (ed.) Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs. Advances in Experimental Medicine & Biology, 141-153. Springer.

Brittain, S.M., Wang, J., Babcock ,J.L., Carmichael, W.W., Rinehart, K.L., and Culver, D.A. 2000. Isolation and characterization of microcystins, cyclic heptapeptide hepatotoxins from a Lake Erie strain of Microcystis aeruginosa. Journal of Great Lakes Research.26:241-249.

Butler N., Carlisle,J., and Linville, R. 2012.Toxicological Summary and Suggested Action Levels to Reduce Potential Adverse Health Effects of Six Cyanotoxins. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento. 119 pp.

Carmichael, W. W. 1981. Freshwater Blue-green Algae (cyanobacteria) Toxins—a Review. In: W. W. Carmichael (ed.) The Water Environment: Algal Toxins and Health. Plenum Press: New York,pp. 1-13. 491 pp.

Carmichael, W.W. 1997. The Cyanotoxins. In: *Advances in Botanical Research*. Academic Press, London. J.A. Callow, (Ed)**47**, 211-255.

Carmichael, W.W. 2001. Reemergence of Cyanobacteria Harmful Algae Blooms in the Great Lakes-the CyanoHABs. The Third Biennial Conference of the Lake Erie Millennium Network.The University of Windsor, Ontario. (oral presentation).

Carmichael, W.W. 2001. Assessment of Blue-green Algal Toxins in Raw and Finished Drinking Water.AWWA Research Foundation. 179 pp.

Carmichael, W.W. 2001. Health Effects of Toxin Producing Cyanobacteria: "The CyanoHABS". Human and Ecological Risk Assessment.7(5):1393-1407.

Carmichael, W.W. 2013. An Overview of Monitoring and Detection Methods for Cyanotoxins Produced by Cyanobacteria Harmful Algae Blooms (CyanoHABs). J. AOAC Int. Proceedings of the Marine and Freshwater Toxins Analysis Third Joint Symposium and Task Force Meeting June 18th - June 22nd 2012, University of Puget Sound, Tacoma, WA (in press).

Carmichael, W.W., Azevedo, M.F.O., An, J.S., Molica, R.J.R., Jochimsen, E.M., Lau, S., Rinehart, K.L., Shaw, G.R., Eagelsham, G.K. 2001. Human Fatalities from Cyanobacteria: Chemical and Biological Evidence for Cyanotoxins. Environmental Health Perspectives. 109 (7):663-668.

Carmichael, W.W**.**, Evans, W.R., Yin, Q.Q., Bell, P. and Moczydlowski, E. .1997. Evidence for Paralytic Shellfish Poisons in the Freshwater Cyanobacterium *Lyngbya wollei* (Farlow ex Gomont) comb.nov. J. Appl. Envir. Microbiol. 63(8): 3104-3110.

Carmichael, W.W., M. Stukenberg. 2006. North American CyanoHABs. In. Codd, G.A., S.M.F.O. Azevedo, S.N. Bagchi, M.D. Burch, W.W. Carmichael, W.R. Harding, K. Kaya, H.C. Utkilen. *CYANONET, a Global Network for Cyanobacterial Bloom and Toxin Risk Management: Initial Situation Assessment and Recommendations. UNESCO/IHP-Paris: Working Series #SC-2005/WS/55.*

Chapman, A. 2010. Cyanobacteria. In Organisms section. AWWA Manual M57, Algae: Source to Treatment. Ch. 5. pp. 125-146.

Chapman, A.D. and C.L. Schelske. 1997. Recent Appearance of *Cylindrospermopsis* (Cyanobacteria) in Five Hypereutrophic Florida Lakes. J. Phycol.33**,**191-195

Chen, J., Xie, P., Li, L., and Xu, J. 2009. First Identification of the Hepatotoxic Microcystins in the Serum of a Chronically Exposed Human Population Together with Indication of Hepatocellular Damage. Toxicological Sciences. 108(1): 81-89.

Chen, H., Burke, J. Mosindy, T. Fedorak, P., and Prepas, E. 2009. Cyanobacteria and microcystin-LR in a complex lake system representing a range in trophic status: Lake of the Woods, Ontario, Canada. Journal of Plankton Research. 31(9):993-1008.

Chorus, I., (ed.) .2012. Current approaches to Cyanotoxin risk assessment, risk management and regulations in different countries. Federal Environment Agency (Umweltbundesamt). Germany. 151 pp.http://www.umweltdaten.de/publikationen/fpdf-l/4390.pdf

Chorus, I., and Bartram, J. (eds.), 1999. Toxic Cyanobacteria in Water: A Guideto Their Public Health Consequences, Monitoring and Management. World HealthOrganization, E&FN Spon, Routledge, London.
http://www.who.int/water_sanitation_health/resourcesquality/toxicyanbact/en/

Codd, G.A., S. Bell, K. Kaya, C. Ward, K. Beattie, and J. Metcalf. 1999. Cyanobacterial toxins, exposure routes and human health. European Journal of Phycology, 34: 405-415.

Codd, G.A., Morrison, L.F., and Metcalf, J.S. 2005. Cyanobacterial Toxins: Risk Management for Health Protection. Tox. Appl. Pharm. 203:264-272.

Codd, G.A., S.M.F.O. Azevedo, S.N. Bagchi, M.D. Burch, W.W. Carmichael, W.R. Harding, K.Kaya, H.C. Utkilen. 2006. CYANONET, a Global Network for Cyanobacterial Bloom and Toxin Risk Management: Initial Situation Assessment and Recommendations. IHP-VI Technical Documents in Hydrobiology No. 76. UNESCO, Paris. http://unesdoc.unesco.org/Ulis/cgi-bin/ulis.pl?catno=142557&set=4B9E6519_2_51&gp=&lin=1&ll=1

Cox, P.A., Banack, S.A., Murch, S.J., Rasmussen, U., Tien, G., Bidigare, R.R., Metcalf, J.S., Morrison, L.F., Codd, G.A., and Bergman, B. 2005. Diverse Taxa of Cyanobacteria

Produce Beta-N-Methylamino-L-Alanine, a Neurotoxic Amino Acid.Proc. Natl. Acad. Sci.102, 5074-78

Cronberg, G., and Annadotter, H. 2006. Manual on Aquatic Cyanobacteria. ISSHA. Denmark. 106 pp.

Devlin, J.P., Edwards, O.E., Gorham, P.R., Hunter, N.R., Pike, R.K., and Stavric, B. 1977. Anatoxin-a, a Toxic Alkaloid From*Anabaena flos-aquae* NRC-44H. Can. J. Chem. 55 (177), 1367-71.

Dodds, W.K., Bouska, W.W., Eitzmann, J.L., Pilger, T.J., Pitts, K.L. 2009. Eutrophication of U.S. freshwaters: analysis of potential economic damages. Environ. Sci. Technol. 43:12–19.

Falconer, I.R. and Humpage, A.R. 1996 Tumour promotion by cyanobacterial toxins Phycologia, 35(6): 74-79.

Fortin, N., Aranda-Rodriguez, R., Jing H., Pick, F., Bird, D. and Greer, C. 2010. Detection of Microcystin-Producing Cyanobacteria in Missisquoi Bay, Quebec, Canada, Using Quantitative PCR, Appl Environ Microbiol. 76(15): 5105–5112.

Francis, G. 1878. Poisonous Australian Lake. Nature. May 2, 1878

Frey, J. S. 2004. Culture, Isolation and Analysis of Anatoxin-a-, Cylindrospermopsin- and Microcystin-Producing Cyanobacteria in the St. Johns River Water Management District, Florida. M.S. Thesis, Department of Biological Sciences, Wright State University, Dayton, Ohio.

Funari, E., and Testai, E. 2008. Human Health Risk Assessment Related to Cyanotoxins Exposure. Crit. Rev. Toxicol. 38:97-125.

Giannuzzi, L., Sedan, D., Echenique, R., and Andrinolo, D. 2011.An Acute Case of Intoxication with Cyanobacteria and Cyanotoxins in Recreational Water in Salto Grande Dam, Argentina. Mar. Drugs. 9, 2164-2175.

Giddings, M., Aranda-Rodriguez, R., Yasvinski, G., Watson, S.B., and Zura-well, R. 2012.Canada: Cyanobacterial Toxins; Drinking and Recreational Water Quality Guidelines. In Chorus, I. (ed.) ,2012. Current approaches to Cyanotoxin risk assessment, risk management and regulations in different countries. Federal Environment Agency (Umweltbundesamt). Germany. Pp. 29-39.http://www.umweltdaten.de/publikationen/fpdf-l/4390.pdf

Gilroy, D. J., Kauffman, K. W., Hall, R. A., Huang, X., and Chu, F. S. 2000. Assessing Potential Health Risks from Microcystin Toxins in Blue-green Algae Dietary Supplements.Environ. Health Perspect. 108(5):,435-439.

Graham, J.L., Loftin, K.A., and Kamman, N. 2009. Algal Toxins-Monitoring Recreational Waters. Lakeline. Summer edition. North American Lakes Management Society. Pp. 18-24.

Grosse, Y., Baan, R., Straif, K., Secretan, B., El Ghissassi, F., and Cogliano, V., 2006.Carcinogenicity of Nitrate, Nitrite, and Cyanobacterial Peptide Toxins. The Lancet. Vol. 7. August 2006. http://oncology.thelancet.com

Harada, K. 1996. Chemistry and detection of microcystins. In: "Toxic Microcystis" M. F. Watanabe,K. Harida, W. W. Carmichael, and H. Fujiki, Eds., CRC Press, Boca Raton, FL, pp. 103-148.

Harrness, 2005. Harmful Algal Research and Response: A National Environmental Science Strategy 2005-2015. Ramsdell, J.S., D.M. Anderson and P.M. Gilbert (Eds.), Ecological Society of America, Washington DC, 82 pp**.**

Hawkins, P.R., Runnegar, M.T.C., Jackson, A.R.B. and Falconer, I.R. 1985. Severe hepatotoxicity caused by the tropical cyanobacterium (blue-green alga)*Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Raju isolated from a domestic water supply reservoir. Appl. Environ. Microbiol., 50,1292-1295.

Heiskary, S., and Lindon, S. 2009. Microcystin in Minnesota Lakes. Lakeline. Summer 2009. Pp. 25-30.

Hereman, T.C., and Bittencourt-Oliveira, M. de Carmo. 2012. Bioaccumulation of Microcystins in Lettuce. J. Phycol. 48: 1535-1537.

Hitzfeld, B. C., Hoeger, S. J., and Dietrich, D. R. 2000. Cyanobacterial Toxins: Removal During Drinking Water Treatment, and Human Risk Assessment. Environ. Health Perspect. 108(S1):113-122.

Hoff, B., Thomson, G.T., and Graham, K. 2007. Neurotoxic cyanobacterium (blue-green alga) toxicosis in Ontario. Can. Vet. J. 48(2): 147.

Holland, T., St. Amand, A., and Good, G. 2006.A Case Study of a Successful Response to a Potentially Toxic Cylindrospermopsis raciborski Bloom.Lakeline Summer Edition. Pp. 52-56.

Hotto, A.M., Satchwell, M.F., and Boyer, G.L. 2007. Molecular characterization of potential microcystin-producing cyanobacteria in Lake Ontario embayments and nearshore waters. Appl. Environ. Microbiol.**73**: 4570-4578.

Hudnell, K. ed. 2008.Cyanobacterial Harmful Algal Blooms.Proceedings of the Interagency, International Symposium on Cyanobacterial Harmful Algal Blooms. RTP North Carolina, Sept. 2005. Advances in Experimental Medicine & Biology. Springer

Science. Vol. 619, 948 pp.
http://www.epa.gov/cyano_habs_symposium/monograph.html

Humpage, A.R., Rositano, J., Bretag, A.H., Brown, R., Baler, P.D., Nicholson, B.C. and Steffensen, D.A. 1994. Paralytic shellfish poisons from Australian cyanobacterial blooms. Aust. J. Mar. Freshwater Res., 45(5), 761-771.

Impacts de l'exposition humaine aux cyanobactéries et à leurs toxines : amélioration des connaissances et de la gestion des épisodes de fleurs d'eau de cyanobactéries au Québec. 2012. Lévesque, B. et al. Fonds de Rechereche Nature et Technologies. Forum de transfert sur les cyanobactéries.Hôtel Palace Royal 775, avenue Honoré-Mercier Québec (Québec) 9 Février 2012.

Jochimsen, E.M., Carmichael, W.W., An, J.S., Cardo, D.M., Cookson, S.T., Holmes, C.E.M., Antunes, M.B. de C., Filho, D.A. de Melo, Lyra, T.M., Burreto, V.S.T., Azevedo, S.M.F.O. and Jarvis, W.R. 1998. Liver Failure and Death Following Exposure to Microcystins at a Hemodialysis Center in Brazil. New Eng. J. Medicine. March 26, 1998, 338(13): 873-878.

Komarek and Anagnostidis. 1998. Vol. 19/1 Cyanoprokaryota. 2. Chroococcales. Spektrum.

Komarek and Anagnostidis. 2005. Vol. 19/2. Cyanoprokaryonta. 2. Oscillatoriales. Spektrum

Kotak, B.G., Zurawell, R.W. 2007. Cyanobacteria toxins in Canadian freshwaters: A review. Lake Reserv. Manage. 23:109-122.

Lagos, N., Onodera, H., Zagatto, P.A., Andrinolo, D., Azevedo, S.M.F.O., and Oshima, Y. 1999.The First Evidence of Paralytic Shellfish Toxins in the Freshwater Cyanobacterium *Cylindrospermoposis raciborskii,* Isolated From Brazil. Toxicon. 37(11858), 1359-73

Lajeunesse, A., Segura,P.A., Gélinas, M., Hudon, C., Thomas, K., Quilliam, M.A., and Gagnona, G. 2012.Detection and confirmation of saxitoxin analogues in freshwater benthic Lyngbya wollei algae collected in the St. Lawrence River (Canada) by liquid chromatography–tandem mass spectrometry. J. Chromat. A. 1219: 93-103.

Lee, E.L, Pang X.L. 2013. New strains of norovirus and the mystery of viral gastroenteritis epidemics. Canadian Medical Association Journal. 185(16): 1381-2.

Lévesque, B., Gervais, M-C., Chevalier, P., Gauvin, D., Anassour-Laouan-Sidic, E., Gringas, S., Fortin, N., Brisson, G., Greer, C., Birde, D. 2014. Prospective study of acute health effects in relation to exposure to cyanobacteria Sci. Tot. Environment 466–467: 397–403.

Li, Y., Chen, J.A., Zhao, Q., Pu, C., Qiu, Z., Zhang, R., and Shu, W.Q. 2011. A Cross-Sectional Investigation of Chronic Exposure to Microcystin in Relationship to Childhood Liver Damage in the Three Gorges Reservoir Region, China. Environmental Health Perspectives. 119(10): 1483-1488.

Loftin, K.A., Graham, J.L., Meyer, M.T., Ziegler, A.C., Dietze, J.E. Holdsworth, S., Tarquinio, E. 2008. ,Preliminary Assessment of Cyanotoxin Occurrence in Lakes and Reservoirs in the United States, 2008, National Water Quality Monitoring Conference, Atlantic City, NJ, May, 21, 2008.(ppt)

Long, E.G., White, E.H.,Carmichael, W.W., Quinlisk, P.M., Raja, R., Swisher, B.L., Daugharty, H. and Cohen, M.T. 1991. Morphologic and Staining Characteristics of a Cyanobacterium-like Organism Associated with Diarrhea. The J. Infectious Diseases.164: 199-202.

Lopez, C.B., Jewett, E.B., Dortch, Q., Walton, B.T., Hudnell, H.K. 2008. Scientific Assessment of Freshwater Harmful Algal Blooms. Interagency Working Group on Harmful Algal Blooms, Hypoxia, and Human Health of the Joint Subcommittee on Ocean Science and Technology. Washington, DC.http://www.cop.noaa.gov/stressors/extremeevents/hab/habhrca/FreshwaterReport_final_2008.pdf

Makarewicz, J.C., Boyer,G.L., Lewis, T.L., Guenther, W., Atkinson, J., and Arnold, M. 2009. Spatial and temporal distribution of the cyanotoxin microcystin-LR in the Lake Ontario ecosystem: Coastal embayments, rivers, nearshore and offshore, and upland lakes. J. Great Lakes Research. 35:83-89.

Meriluoto, J. and Codd, G.A. ed. 2005. Cyanobacterial Monitoring and Cyanotoxin Analysis. Åbo Akademi Univ Press. Åbo, Finland.

Milligan, A.J. 2009. Cyanobacterial Toxins Impact Crops and Accumulateto Levels Considered Unsafe for Consumption.(Unpublished review).

MMWR CDC. 1991. Epidemiologic Notes and Reports Outbreaks of Diarrheal Illness Associated with Cyanobacteria (Blue-Green Algae)-Like Bodies -- Chicago and Nepal, 1989 and 1990. May 17, 1991 / 40(19);325-327.

MMWR Recomm Rep 2011. Updated norovirus outbreak management and disease prevention guidelines. 60: 1-18.

Murch, S.J., Cox, P.A., Banack, S.A., Steele, J.C., and Sacks, O.W. 2004. Occurrence of Beta-Methylamino-L-Alanine (BMAA) in Als/Pdc Patients From Guam. Acta. Neurol. Scand. 110, 267-69

Murphy, T.P., Irvine, K., Guo, J., Davies, J., Murkin, H., Charlton, M., Watson, S.B. 2003.New Microcystin Concerns in the Lower Great Lakes. Water Qual. Res. J. Can. 38(1):127-140.

O'Donoghue, J. G. and Wilton, G.S.1951. Algal poisoning in Alberta.Can. J. Comp. Med. 15(8): 193-198.

Ohtani, I., Moore, R.E. and Runnegar, M.T.C. 1992.Cylindrospermopsin, a potent hepatotoxin from the blue-green alga *Cylindrospermopsis raciborskii.*J. Amer. Chem. Soc., 114, 7941-7942.

Olson, T. A. (1960). Water poisoning — a study of poisonous algae blooms in Minnesota. Am. J. Pub. Health 50:883-884.

Onodera, H, Oshima, Y, Henriksen, P, & Yasumoto, T. (1997) Confirmation of Anatoxin-a(s), in the Cyanobacterium *Anabaena lemmermannii,* as the Cause of Bird Kills in Danish Lakes.Toxicon.35(4098), 1645-48.

Orr, P.T. and Schneider, P.M. 2006. Toxic Cyanobacteria Risk Assessment Reservoir Vulnerability and Water Use Best Practice. SEQWater, Brisbane, Australia. 61 pp.

Ouellette, A.J.A., Handy, S.M., and Wilhelm, S.W. 2006. Toxic *Microcystis* is widespread in Lake Erie: PCR detection of toxin genes and molecular characterization of associated cyanobacterial communities. Microbial Ecology. 51:154-165.

Paerl , H.W. and Otten, T.G. 2013. Harmful Cyanobacterial Blooms: Causes, Consequences, and Controls. Microbial Ecology. Jan. 13, 2013 (in Press).

Paerl, H. 2005. Nutrient and other environmental controls of harmful cyanobacterial blooms along the freshwater-marine continuum.In Hudnell, K. ed. 2005.Cyanobacterial Harmful Algal Blooms. Springer, 2008.Proceedings of the Interagency, International Symposium on Cyanobacterial Harmful Algal Blooms. RTP North Carolina, Sept. 2005. Advances in Experimental Medicine & Biology. Springer Science. Vol. 619.http://www.epa.gov/cyano_habs_symposium/monograph.html

Poste, A., Hecky, R.E., and Guildford, S.J. 2011. Evaluating Microcystin Exposure Risk through Fish Consumption. Envir. Sci. Technol. 45(13): 5806-5811.

Pouria, S., Andrade, A., Barbosa, J., Cavalcanti, R.L., Barreto, V.S.T., Ward, C.J., Preiser, W., Poon, G.K., Neild, G.H., and Codd, G.A. 1998. Fatal Microcystin Intoxication in Haemodialysis Unit in Caruaru, Brazil. Lancet352, 21-26.

Rasmussen, J.P., Monis, P.T., and Saint, C.P., 2007. Early detection of cyanobacterial toxins using genetic methods.AWWA research foundation, CRC for Water Quality and Treatment.

Report of the Phosphorus Reduction Task Force To the Great Lakes Commission. 2012.Priorities for reducing phosphorus loadings and abating algal blooms in the Great Lakes – St. Lawrence river basin: opportunities and challenges for improving Great Lakes aquatic ecosystems.September 2012.

Ressom, R., Soong, F. S., Fitzgerald, J., Turczynowicz, L., El Saadi, O., Roder, D. M., Maynard, T., and Falconer, I. R. 1994.Health Effects of Toxic Cyanobacteria (Blue-Green Algae).Australian Government Publishing Service: Canberra, ACT, Australia. 108 pp.

Rinta-Kanto, J. M., Ouellette, A.J.A., Twiss, M.R., Boyer, G.L., T. Bridgeman, T. and Wilhelm, S.W. 2005.Quantification of toxic Microcystis spp. during the 2003 and 2004 blooms in western Lake Erie using quantitative real-time PCR. Environ. Sci. Technol. **39**: 4198-4205.

Sarma, T.A. 2013a.Cyanobacterial Toxins in Handbook of Cyanobacteria. CRC Press. Taylor and Francis Group,pp. 487-606

Sarma, T.A. Handbook of Cyanobacteria. 2013. CRC Press. Taylor and Francis Group. 802 pp.

Sivonen, K. 2008. Emerging high throughput analysis of cyanobacterial toxins and toxic cyanobacteria. Chapter 24 In *Cyanobacterial Harmful Algae Blooms, State of the Science and Research Needs*. Proceedings of the Interagency, International Symposium on Cyanobacterial Harmful Algal Blooms. RTP North Carolina, Sept. 2005. Hudnell, H.K. (ed.) Advances in Experimental Medicine & Biology. Springer Science. Vol. 619, 948 pp. http://www.epa.gov/cyano_habs_symposium/monograph.html

Soward, T.E. 2011. Evaluation of Cancer from Exposure to Cyanotoxins.Thesis, Master of Public Health Program, Wright State University, Dayton, Ohio. 54 pp.

Steffensen, D. 2007. Economic Costs of Cyanobacterial Blooms. In: K. Hudnell, ed., Cyanobacterial Harmful Algal Blooms. Springer, Advances in Exp. Biol. Vol. 619. Proceedings of the Interagency, International Symposium on cyanobacterial Harmful Algal Blooms, pp. 849-859.

Stewart, I., Webb, P.M., Schluter, P.J., Shaw, G.R. 2006. Recreational and occupational field exposure to freshwater cyanobacteria—a review of anecdotal and case reports, epidemiological studies and the challenges for epidemiologic assessment. EnvironHealth 2006, 5:6.

Stewart, I., Carmicheal, W. and Backer, L. 2011. Toxic Cyanobacteria. In: J. Salendy, ed., Water and Sanitation-Related Diseases and the Environment. Wiley-Blackwell, pp. 95-110.

Svirˇcev,V., Krsti´c, S., Miladinov-Mikov, M., Balti´c, V. and Vidovi´c, M. 2009. Freshwater Cyanobacterial Blooms and Primary Liver Cancer Epidemiological Studies in Serbia. J. Envir. Sci. Health, Part C. 27:36-55.

The State of the Nation's Ecosystems 2008. The H.J. Heinz III Center for Science, Economics and the Environment. Island Press. Washington D.C. 352 pp.

U.S. Lakes Microcystin Dataset with relative probable human health effects assessment based on WHO guidelines. http://water.epa.gov/type/lakes/NLA_data.cfm

Vanderploeg, H.A., Liebig, J.R., Carmichael, W.W., Agy, M.A., Johengen, T.H., Fahnenstiel, G.L., and Nalepa, T.F. 2001. Zebra mussel (*Dreissena polymorpha*) selective filtration promoted toxic *Microcystis* blooms in Saginaw Bay (Lake Huron) and Lake Erie. Canadian Journal of Fisheries and Aquatic Sciences 58(6): 1208-1221.

Watson, S.B. and Boyer, G. 2009. Harmful Algal Blooms (HABs) in the Great Lakes: Current Status and Concerns. In State of the Lakes Ecosystem Conference (SOLEC) 2008. Nearshore Areas of the Great Lakes September 2009. Pp. 78-91.

Watson, S.B., and Boyer, G. 2011. Harmful Algal Blooms Indicator Draft. SOLEC 2011. Oct. 26-27, 2011. Bayfront Convention Center, Erie, PA.pp. http://www.solecregistration.ca/en/indicator_reports.asp

Watson, S.B., Charlton, M.N., Murphy, T., Mamone, T. and Parr, T. 2003. Hamilton Harbour: phytoplankton and algal toxins. Hamilton Harbour Remedial Action Plan Report 2002 Season Burlington, Ont. March 2003..ISSN 1703-4043. pp. 67-80

Weise, G., Drews, G., Jann, B. and Jann, K. 1970. Identification and analysis of a lipopolysaccharide in cell walls of the blue-green algae *Anacystis nidulans*.Archives of Microbiology 71:89–98.

WHO 2003. Guidelines for Safe Recreational Water Environments, Volume 1: Coastal and Fresh Waters. World Health Organization. http://www.who.int/water_sanitation_health/bathing/srwe1/en/

Wilson, A.E., Gossiaux, D.C., Hook, T.O., Berry, J.P., Landrum, P.F., Dyble, J., and Gulldford, S.J. 2008. Evaluation of the human health threat associated with the hepatotoxin microcystin in the muscle and liver tissues of yellow perch (Perca flavescens). Can. J. Fish Aquat.Sci. 65:1487-1497.

Winter, J.G., DeSelias, A.M., Fletcher, R., Heintsch, L., Morley, A., Nakamoto, L. and Utsumi, K. 2011. Algal blooms in Ontario, Canada: Increases in reports since 1994. Lake and Reservoir Management. 27:105-112.

Yu, S-Z. 1989. Drinking Water and Primary Liver Cancer. In: Z-Y. Tang; M-C. Wu, and S-S. Xia, (eds.) Primary Liver Cancer.Springer-Verlag: Berlin & China Acad. Publ.: Beijing, pp. 30-37, Ch. 4. 495 pp.

Zegura, B., Straser, A., and Filipic, M. 2011.Genotoxicity and Potential Carcinogenicity of Cyanobacterial Toxins – a Review. Mutation research. 727:16-41.

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G. Appendices

Appendix A. Selected websites for CyanoHABs from Federal and State/Provincial Agencies

National l (U.S. and Canada)

USEPA
http://water.epa.gov/scitech/swguidance/standards/criteria/nutrients/cyanohabs.cfm

USEPA Great Lakes Monitoring
http://www.epa.gov/glindicators/water/trophicb.html

USGS
http://ks.water.usgs.gov/studies/qw/cyanobacteria/

CDC
http://www.cdc.gov/nceh/hsb/hab/default.htm

NOAA
http://www.glerl.noaa.gov/res/Centers/HumanHealth/habs_resources.html

Health Canada
http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/cyanobacter-eng.php

US/Canada Border States/Provinces CyanoHAB Websites

Illinois
http://www.epa.state.il.us/water/surface-water/blue-green-algae.html

Indiana
http://www.in.gov/idem/algae/

Michigan
http://www.michigan.gov/deq/0,4561,7-135-3313_3677---,00.html

Minnesota
http://www.pca.state.mn.us/index.php/water/water-types-and-programs/surface-water/lakes/blue-green-algae-and-harmful-algal-blooms.html

New York
http://www.health.ny.gov/environmental/water/drinking/bluegreenalgae.htm

Ohio
http://epa.ohio.gov/dsw/hab.aspx

Ontario
http://www.ene.gov.on.ca/environment/en/blog/STDPROD_082497.html

Quebec
http://www.mddefp.gouv.qc.ca/eau/flrivlac/algues-en.htm

Vermont
http://healthvermont.gov/enviro/bg_algae/bgalgae.aspx

Wisconsin
http://dnr.wi.gov/lakes/bluegreenalgae/

Appendix B. Summary of Current Approaches to Cyanotoxin Risk Assessment, Risk Management and Regulations in Different Countries.

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